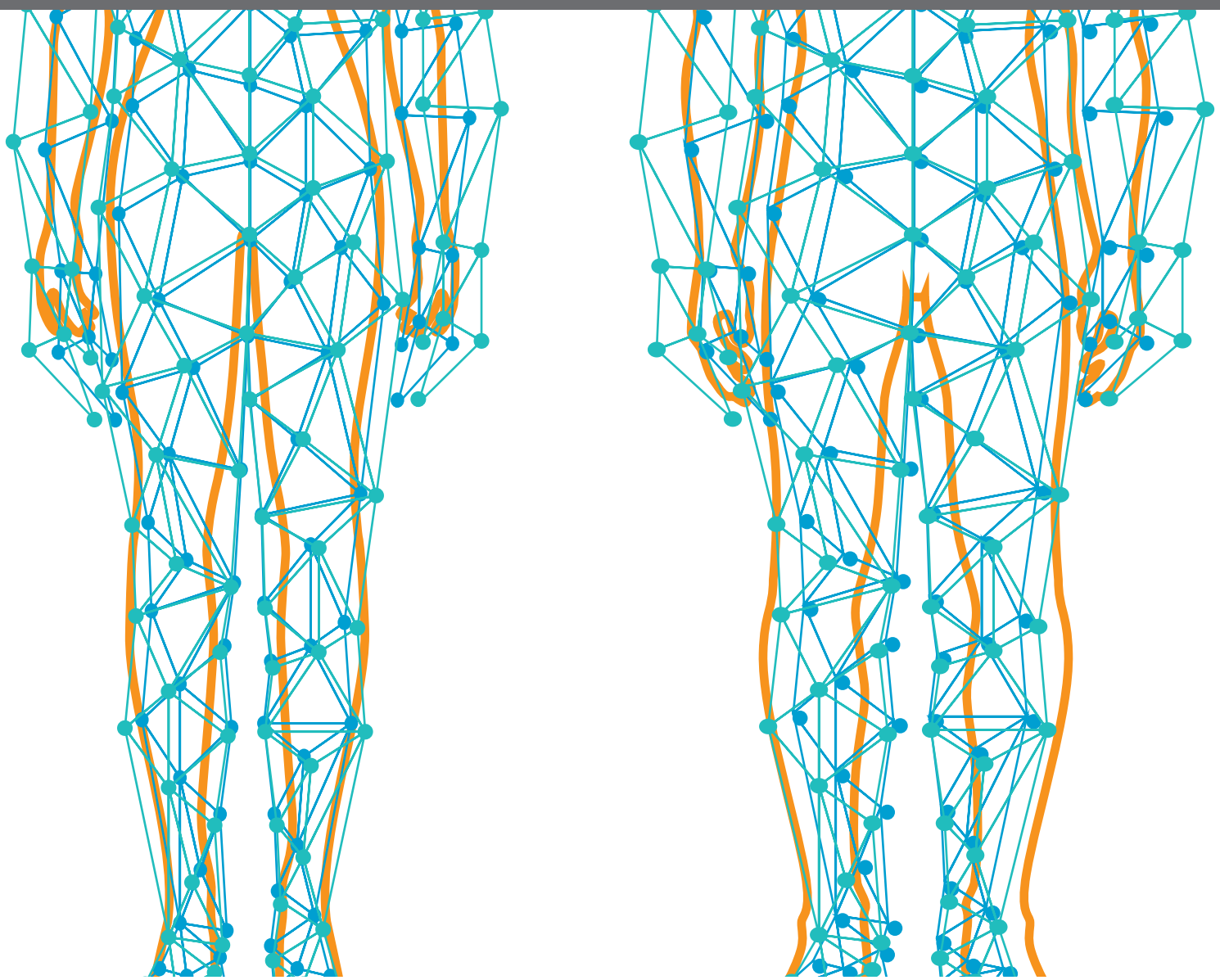
IMMUNOSENESCENCE AND IMMUNOEXHAUSTION IN CHRONIC KIDNEY DISEASE AND RENAL TRANSPLANTATION

EDITED BY: Maria J. Stangou, Asimina Fylaktou,
Milena Ivanova Ivanova - Shivarova and Ioannis Theodorou

PUBLISHED IN: Frontiers in Medicine and Frontiers in Immunology



frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88976-063-3
DOI 10.3389/978-2-88976-063-3

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

IMMUNOSENESCENCE AND IMMUNOEXHAUSTION IN CHRONIC KIDNEY DISEASE AND RENAL TRANSPLANTATION

Topic Editors:
Maria J. Stangou, Aristotle University of Thessaloniki, Greece
Asimina Fylaktou, Hippokration General Hospital, Greece
Milena Ivanova Ivanova - Shivarova, Aleksandrovska University Hospital, Bulgaria
Ioannis Theodorou, Assistance Publique Hopitaux De Paris, France

Citation: Stangou, M. J., Fylaktou, A., Ivanova - Shivarova, M. I., Theodorou, I., eds. (2022). Immunosenescence and Immunoexhaustion in Chronic Kidney Disease and Renal Transplantation. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88976-063-3

Table of Contents

frontiers | Frontiers in Medicine

EDITORIAL
published: 05 April 2022
doi: 10.3389/fmed.2022.874581

Editorial: Immunosenescence and Immunoexhaustion in Chronic Kidney Disease and Renal Transplantation

Maria J. Stangou[1]*, *Asimina Fylaktou*[2], *Milena Ivanova Ivanova-Shivarova*[3] and *Ioannis Theodorou*[4]

[1] Department of Nephrology, Aristotle University of Thessaloniki, Thessaloniki, Greece, [2] Department of Immunology, National Peripheral Histocompatibility Center, Hippokration Hospital, Thessaloniki, Greece, [3] Department of Clinical Immunology, University Hospital Aleksandrovska, Medical University, Sofia, Bulgaria, [4] Assistance Publique Hopitaux De Paris, Paris, France

Keywords: renal function, immunosenescence and exhaustion, kidney transplantation, gut microbiome, glomerulonephritis, hemodialysis

Editorial on the Research Topic

Immunosenescence and Immunoexhaustion in Chronic Kidney Disease and Renal Transplantation

The Immune System (IS) and Kidney function are closely and interactively connected (1). Dysregulation of the IS, as it occurs in systemic autoimmune diseases, may affect kidneys through several pathways, including immune complex deposition, signaling transduction pathways or complement activation, leading to an excessive variety of glomerular and interstitial disorders (2, 3). Moreover, chronic kidney disease (CKD) *per se*, characterized as an "inflamm-aging" condition, seems to affect immune integrity, in a way similar to aging process; mainly directed to adaptive immunity, leading to a shift of lymphocytes toward senescent and exhausted phenotypes (4, 5). Clinical consequences, including increased cardiovascular risk, susceptibility to infections and reduced response to immunization, are critical (6, 7). Following kidney transplantation (KT), renal function is reinstated; yet initiation of immunosuppressive treatment may simply change the scene of IS disturbances.

Apparently, the interaction between IS and kidneys is multifaceted and extremely important in many aspects (8). This special issue aimed to gather participation of investigators to present their latest findings upon immune-senescent and exhaustion phenomena relative to renal function. A group of eminent authors participated, with nine papers, four original research articles, four reviews, and one case report.

The gut microbiome may act as a bridge between kidneys, aging and IS disorders (9). Microbiota evolves normally from newborn to elderly, as a result of epigenetic mechanisms, environmental factors, personal habits, nutrition, etc. Recent studies have implicated microbiota in the pathogenesis of primary glomerular diseases, such as IgAN and membranous nephropathy (8). In this issue, the review from Stavropoulou et al., describes that CKD side-effects, such as metabolic disorders, dyslipidemia, oxidative stress, converging to gut dysbiosis, may generate the "inflammaging" status, leading to immune senescent alterations.

Predicting the progression of a glomerular disease is a useful tool for clinicians in order to determine treatment approach. Papasotiriou et al., presented the ability of survival models, recently designed to predict long term outcome of IgA Nephropathy (IgAN), the most common glomerulonephritis worldwide and common cause of end stage renal disease (ESRD). The authors

Edited and reviewed by:
Minnie M. Sarwal, University of California, San Francisco, United States

***Correspondence:**
Maria J. Stangou
mstangou@auth.gr

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *12 February 2022*
Accepted: *01 March 2022*
Published: *05 April 2022*

Citation:
Stangou MJ, Fylaktou A, Ivanova-Shivarova MI and Theodorou I (2022) Editorial: Immunosenescence and Immunoexhaustion in Chronic Kidney Disease and Renal Transplantation. Front. Med. 9:874581. doi: 10.3389/fmed.2022.874581

showed that predictive models may overestimate the risk probability, however they were accurate to distinguish high risk IgAN patients.

As the adaptive immunity is predominantly affected in CKD, resulting in lymphocyte phenotypic changes, Duni et al., analyzed specific lymphocyte subsets, including B cells, CD14++CD16+ monocytes, Natural Killer cells (NKs) and regulatory T lymphocytes (Tregs), in peritoneal dialysis (PD) patients. B cell lymphopenia, together with increased CD14++CD16+ monocytes and NK cells were closely associated with the presence of CVD, but also there was a direct association of these lymphocyte subtypes with adequacy of PD method and fluid balance.

Apart from the increased incidence of cardiovascular disease, CKD patients have a phenotype of premature aging, characterized by frailty, muscle wasting, and osteoporosis. The shift toward senescent T cell subtypes, as the result of a premature thymic involution, is compensated by homeostatic expansion of highly differentiated memory T lymphocytes (10). Potential therapeutic interventions to prevent or even reverse ESRD-related premature immune-senescence are described by Ducloux et al., and include increased physical activity and dietary interventions to modulate gut microbiota and reduce levels of protein bound uremic toxins (p-cresol, p-cresyl sulfate). Administration of growth hormone is a safe procedure, which leads to increased IGF1 levels and may reverse thymic involution. The use of median cut-off or vitamin E-coded dialyzers are preferred in hemodialysis, although PD seems more advantageous. KT, although essentially restores renal function, it cannot reverse thymus involution and its effect on recovering adaptive immunity is still under investigation.

Increased risk of infection in ESRD has become more profound the last 2 years, during the COVID-19 pandemic. Co-morbid conditions, such as CKD and organ transplantation, are associated with the highest mortality risk from COVID-19 infection. Betjes presents pathogenic mechanisms of severe COVID-19 infection in CKD patients and the role of immune senescence in the evolution of the disease. The contracted TcR repertoire in naïve T lymphocytes and reduced numbers of plasmocytoid dendritic cells, in CKD patients, may have a profound negative effect on control of viral infections. Moreover, CKD and elderly patients are characterized by increased proportion of CD4+CD28 null cells, advanced differentiated cells, highly activated and poorly controlled, responding with a cytokine storm, responsible for lung parenchyma damage.

In the study of Weiger et al., response to BNT162b2 vaccination was significantly lower in HD patients compared to healthy controls. After the first dose almost 70% of patients were seronegative. Anti-spike IgG levels were increased only after the second dose, and almost disappeared over the following 4 months. These findings describe a late response to vaccination in HD patients, with limited duration, and they advocate for the specific management of these patients during COVID-19 pandemic with reinforced vaccination schedules.

KT is undoubtedly the treatment of choice for CKD, although this usually comes after protracted periods undergoing on dialysis. Patients are usually exposed to foreign HLAs, which are processed into smaller peptides loaded onto HLA class II and expressed on antigen presenting cells. After recognition of the above T cell epitopes (TEs), naïve CD4+ T lymphocytes are differentiated into donor-reactive memory cells, responsible for the early de novo DSA (dnDSA) formation after transplantation. Tomosugi et al., proved that evaluation of shared TEs by the *in silico* assay using the PIRCHE-II algorithm, can estimate donor-reactive memory CD4+ T cells and predict the risk of early dnDSA formation after KT.

Phenotypic markers of lymphocytes (CD3, CD4, CD8, CD19, CD56) monocytes (CD14, CD16, CD86, and CD54), and endothelium-derived microvesicles (MV) (annexin V+CD31+CD41−) were estimated in KT patients and compared to healthy controls and CKD patients, by Ceprian et al. Surprisingly, B-cell lymphopenia together with the increased numbers of T-cytotoxic lymphocytes and activated monocytes persisted after KT, and correlated negatively with MVs. Findings of the present study were novel and may explain the persistent adverse outcome of CVD despite KT.

B-cell lymphopenia and acquired agammaglobulinemia further deteriorates after reaching ESRD and is complicated by recurrent infections, however, as Pavlakou et al., presented that administration of IVIG may be curative and can safely be continued even after KT, together with immunosuppression.

We believe the present issue will be a valuable implement to promote further investigation in order to understand the Immune System-Kidney axis.

AUTHOR CONTRIBUTIONS

MS: conceptualization and writing the original draft. AF, MI-S, and IT: conceptualization, writing—review, and editing. All authors approved the submitted version.

ACKNOWLEDGMENTS

We would like to express our gratitude to all the authors who presented their latest findings and all the reviewers for their valuable contribution to improve the quality of accepted papers.

REFERENCES

1. Lioulios G, Fylaktou A, Papagianni A, Stangou M. T cell markers recount the course of immunosenescence in healthy individuals and chronic kidney disease. *Clin Immunol.* (2021) 225:108685. doi: 10.1016/j.clim.2021.108685
2. Bantis K, Stangou M, Kalpakidis S, Hatziadamou M, Daikidou DV, Lioulios G, et al. Systemic complement activation in anti-neutrophil cytoplasmic antibody-associated vasculitis and necrotizing glomerulonephritis. *Nephrology (Carlton).* (2021) 26:30–7. doi: 10.1111/nep.13747
3. Bellur SS, Roberts ISD, Troyanov S, et al. Reproducibility of the Oxford classification of immunoglobulin A nephropathy, impact of biopsy scoring on treatment allocation and clinical relevance of disagreements: Evidence from the VALidation of IGA study cohort. *Neph Dial Transplant.* (2019) 34:1681–90. doi: 10.1093/ndt/gfy337

4. Sampani E, Stangou M, Daikidou DV, Nikolaidou V, Asouchidou D, Dimitriadis C, et al. Influence of end stage renal disease on CD28 expression and T-cell immunity. *Nephrology (Carlton).* (2021) 26:185–96. doi: 10.1111/nep.13784
5. Sampani E, Daikidou DV, Lioulios G, Xochelli A, Mitsoglou Z, Nikolaidou V, et al. CD28null and regulatory T cells are substantially disrupted in patients with end-stage renal disease due to diabetes mellitus. *Int J Mol Sci.* (2021) 22:2975. doi: 10.3390/ijms22062975
6. Lioulios G, Stangou M, Sarafidis PA, Tsouchnikas I, Minasidis I, Vainas A, et al. Chronic therapy with sucroferric oxyhydroxide does not affect iron and anemia markers in dialysis patients. *Blood Purif.* (2020) 49:440–7. doi: 10.1159/000505432
7. Ducloux D, et al. ESRD-associated immune phenotype depends on dialysis modality and iron status: clinical implications. *Immun Ageing.* (2018) 15:16. doi: 10.1186/s12979-018-0121-z
8. Ivanova M, Creary LE, Al Hadra B, Lukanov T, Mazzocco M, Sacchi N, et al. 17th IHIW component "Immunogenetics of Ageing" - New NGS data. *Hum Immunol.* (2019) 80:703–13. doi: 10.1016/j.humimm.2019.07.287
9. Rodrigues FG, Ormanji MS, Heilberg IP, Bakker SJL, de Borst MH. Interplay between gut microbiota, bone health and vascular calcification in chronic kidney disease. *Eur J Clin Invest.* (2021) 51:e13588. doi: 10.1111/eci.13588
10. Betjes MGH, Langerak AW, Klepper M, Litjens NHR. A very low thymus function identifies patients with substantial increased risk for long-term mortality after kidney transplantation. *Immun Ageing.* (2020) 17:4. doi: 10.1186/s12979-020-00175-z

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

CASE REPORT
published: 12 March 2021
doi: 10.3389/fmed.2021.665475

Case Report: Kidney Transplantation in a Patient With Acquired Agammaglobulinemia and SLE. Issues and Challenges

*Paraskevi Pavlakou[1], Marios Papasotiriou[1], Theodoros Ntrinias[1], Alexandra Kourakli[2], Adamantia Bratsiakou[1], Dimitrios S. Goumenos[1] and Evangelos Papachristou[1]**

[1] Department of Nephrology and Renal Transplantation, University Hospital of Patras, Achaia, Greece, [2] Hematology Division, Department of Internal Medicine, University Hospital of Patras, Achaia, Greece

Lupus nephritis in the context of Systemic Lupus Erythematosus (SLE) is characterized by an unpredicted course with remissions and flare-ups. Among others, it remains a significant cause of end-stage kidney disease (ESKD) in relatively young patients. Therapeutic regimens with newer immunosuppressive agents have been introduced in order to control SLE clinical manifestations more efficiently and limit organ damage induced by immune complex formation and sustained inflammation. Treatment is usually long-term, and the cumulative impact of immunosuppression is expressed through the increased frequency of infections and neoplasms. However, if the observed immunity dysregulation is secondary and pharmaceutically induced or there is a pre-existing, primary immunodeficiency that shares common pathogenetic pathways with SLE's autoimmunity is not always clear. Herein, we present the case of a 39-year-old woman, that reached ESKD due to lupus nephritis. After an upper respiratory cytomegalovirus (CMV) infection and concomitant CMV reactivations the investigation revealed significant immunodeficiency. Not long after the initiation of intravenous immunoglobulin (IVIG) administration, patient received a cadaveric kidney transplant. IVIG was continued along with standard immunosuppression so that both recurrent infections and allograft rejection are avoided. Patient is closely monitored, and her post-transplant course is remarkably satisfying so far. ESKD patients with immunodeficiency syndromes should not be excluded by definition from kidney transplantation.

Keywords: kidney transplantation, immunodeficiency, systemic lupus erythematosus, hypogammaglobulinemia, intravenous immunoglobulin

OPEN ACCESS

Edited by:
Maria J. Stangou, Aristotle University of Thessaloniki, Greece

Reviewed by:
Aikaterini Lysitska, Papageorgiou General Hospital, Greece
Smaragdi Marinaki, Laiko General Hospital of Athens, Greece

****Correspondence:***
Evangelos Papachristou
epapadoct@hotmail.com

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *08 February 2021*
Accepted: *22 February 2021*
Published: *12 March 2021*

Citation:
Pavlakou P, Papasotiriou M, Ntrinias T, Kourakli A, Bratsiakou A, Goumenos DS and Papachristou E (2021) Case Report: Kidney Transplantation in a Patient With Acquired Agammaglobulinemia and SLE. Issues and Challenges. Front. Med. 8:665475. doi: 10.3389/fmed.2021.665475

INTRODUCTION

Systemic Lupus Erythematosus and Autoimmunity

Lupus nephritis remains a significant cause of end-stage kidney disease (ESKD). Despite the improvement in renal survival during the past decades due to new therapies, the 5-year incidence of ESKD in patients with lupus nephritis is 11% (1). Lupus clinical course is characterized by periods of remissions and unpredicted flareups that dictate the need and even the increase of immunosuppressive treatment in order to control symptoms. The cumulative impact of immunosuppression on patients is reflected on the incidence of infections (2) and even

neoplasms (3) on the long-term, making clear the need for minimization of toxicity. Nevertheless, the dysregulations of immunity that are present are related to the pathophysiology of lupus with emphasis on coexisting genetic variations, lymphopenia and hypocomplementemia aggravating further the susceptibility to infections (4).

Systemic lupus erythematosus (SLE) is a systemic multi-organ chronic disease with genetic predisposition and environmental triggering that lead to the production of autoantibodies against nuclear antigens which are responsible for the disease manifestations. Shared genetic pathways determine the complex interplay between SLE and immunity disorders (4, 5). The underlying deficiency in complement components, the defective immunoglobulin synthesis (partial deficiencies in IgA and IgM mostly) and/or the co-existence of granulomatous or other autoimmunity disorders (i.e., Wiskott-Aldrich syndrome, autoimmune lymphoproliferative syndrome etc) are the main parameters of clinically expressed immunodeficiency and SLE (6). Under these circumstances, there is a constant activation of the immune system against self-antigens, a dysregulated immune complex formation and degradation that lead to unpredicted tissue damage enhancing further any pre-existing tendency toward autoimmunity and SLE as well (4, 5).

Immunodeficiency Syndromes

The range of immunodeficiency syndromes (IS) is wide and consists of primary forms with genetic predisposition and secondarily induced immunity dysregulations that lead to hypogammaglobulinemia and frequent infections (7). Diagnosis demands broad investigation and exclusion of secondary causes. Despite efforts and consensus reports on diagnostic criteria for IS there are overlapping cases that do not fall into one category in specific, termed unspecified hypogammaglobulinemia (8) and mirror the complexity in diagnosing and classifying IS (8, 9). The exact prevalence of immunodeficiency syndromes is not known as the field is currently under study but secondary forms of IS are adding up making the interest for the establishment of a classification even greater (10). Secondary immunodeficiency has been described in patients suffering from hematologic malignancies (lymphoma, multiple myeloma) as well as those under immunosuppressants. A special group of patients are solid organ transplant patients who are likely to develop hypogammaglobulinemia post transplantation but especially those with pre-existing gamma globulin dysregulations and an intention to receive an allograft plus immunosuppressants. As these patients are highly prone to infections and the aetiologic treatment of secondary IS with immunosuppressants' withdrawal is not an option, close monitoring and proper translation of laboratory parameter results is a possible strategy. For example, low levels of complement components indicate an increased risk for bacterial infections while enzyme-linked immune absorbent spot (ELISPOT) and flow cytometry low response of anti-CD8 lymphocytes to cytomegalovirus (CMV) antigens reflect increased probability for CMV infection (11).

B-cell depleting therapies are established as a highly effective treatment option not only in lymphoproliferative disorders but also in collagen inflammatory diseases. However, one cannot avoid recognizing the induced rise in the rate of infections that complicate patients' course that may even be fatal (12), in the context of secondary IS.

The main axis of treatment in patients with IS is the management of infections and their prevention through prophylactic antibiotics and antivirals. The substitution of gamma globulins is another option that seems to benefit patients with significantly low levels of IgG (<4 g/L), recurrent infections with failure of prophylactic antibiotics and immunity disorders namely failure in post-exposure immunization while the route of administration (subcutaneous or intravenous) doesn't affect the effect of the regimen (13).

Case Report

A 39-year-old female patient was referred to our Nephrology Department during late 2009. She was newly diagnosed with SLE after a spontaneous miscarriage during the second trimester of pregnancy. Due to significant proteinuria and microscopic hematuria a kidney biopsy was performed which revealed crescentic focal segmental glomerulonephritis [class III lupus nephritis with crescents according to WHO classification (14)]. She was initially managed with a combined therapeutic regimen of cyclophosphamide, corticosteroids and azathioprine which was modified due to poor response, to mycophenolic acid with the addition of rituximab. However, despite the absence of extra-renal manifestations of lupus, kidney function gradually deteriorated and patient reached ESKD about 4 years after the initiation of immunosuppression. Peritoneal dialysis was the type of renal replacement therapy that was initiated with the concomitant administration of hydroxychloroquine and low dose methylprednisolone.

Shortly after the induction of peritoneal dialysis, the patient presented with fever for the first time. All possible infectious causes were investigated including peritonitis, catheter related infection and a lupus flair, nevertheless no definite diagnosis was reached, and she was managed with empiric antibiotic treatment that led to full recovery. During the next 12 months she had 3 more hospitalizations with fever as main cause of admission and all workups were inconclusive except her last admission when high CMV viral load with 157.000 copies/mL was found. Further investigation revealed remarkably low serum immunoglobulin levels and five pulses of intravenous immunoglobulin were administered. In this direction, supplementary investigation for hypoglobulinemia was performed with repetitive measurement of immunoglobulin levels after a symptom free period which confirmed earlier findings with low IgA, IgG and IgE levels but slightly elevated IgM levels (**Table 1**). Then on, patient had no major complications apart from two to three episodes of upper respiratory infections and acute tonsilitis each year. Evaluating both clinical course and laboratory findings the monthly administration of intravenous immunoglobulin was initiated as acquired immunodeficiency was the most likely cause. The regimen was well tolerated.

In June 2020, the patient received a cadaveric kidney transplant. The compatibility analysis revealed two shared HLA class I antigens and the donor was CMV IgG positive. Immunosuppression regimen included basiliximab 25 mg on

day 0 and 4, mycophenolic acid 540 mg twice daily, tacrolimus with trough levels of 8 ng/ml directly post-operatively and methylprednisolone as well as prophylactic treatment with trimethoprim-sulfamethoxazole and valganciclovir. There was direct allograft function postoperatively and no need for renal replacement therapy. The only remarkable manifestation post-transplant was a lymphocele that was surgically managed with a peritoneal window drainage.

Treatment with IVIG was continued seamlessly and a change in immunoglobulin profile was noticed (**Table 2**). In more detail, IgG CMV antibodies that were absent even after the resolution of her former CMV infection were detected. Of note, our patient had also failed to acquire immunity for hepatitis B after vaccination, but HBV surface antibodies were now present. Peripheral blood immunophenotyping with flow cytometry appointed an almost absent CD20+ lymphocytes count (0.11%, 2 cells/μl).

DISCUSSION

Kidney Transplantation, Immunosuppression and Viral Prophylaxis

Successful solid organ transplantation equals to adequate suppression of recipient's immunity response, so that both self-tolerance and a well-functioning allograft are achieved. The key target for immunosuppressive treatments in solid organ transplantation is the major histocompatibility complex (MHC) or HLA complex. When donor's HLA molecules are presented on antigen-presenting cells (APCs), they are recognized by recipient's MHC as foreign antigens giving rise to an immunity response with T-lymphocytes activation (15). T cell-mediated allorecognition and response, if not halted, is clustering with secondary signals, cytokine production and detrimental effects for the allograft, leading to rejection. Despite described efforts for self-tolerance achievement with minimization or early immunosuppression withdrawal especially in pediatric transplant patients (16), successful kidney transplantation is interconnected with a regimen that consists of two or more often three immunomodulatory agents.

Along with maintenance immunosuppression, prophylactic treatment against CMV with valganciclovir has proven efficacy especially in CMV-IgG negative patients receiving a transplant from CMV-IgG positive donor, who are regarded high risk for CMV infection and invasive disease. Treatment with valganciclovir for 3 months prevents CMV disease and ensues protection against super-infections such as herpes simplex virus while it can lead in medium and low-risk patients to lower incidence of acute rejection (17). Nevertheless, in high-risk seronegative kidney transplant recipients prophylaxis with valganciclovir is recommended for a 6 month period post-transplant (18). An alternative strategy to avoid CMV infection in transplantation is pre-emptive treatment with CMV-specific super-immune globulin, for 3 days directly postoperatively. Results from a meta-analysis in more than 5,000 solid organ transplant patients, show no superiority between valganciclovir prophylaxis and pre-emptive therapy regarding CMV disease prevention, organ rejection and superinfections. Valganciclovir was associated with more episodes of delayed CMV infections especially in seronegative recipients, while hematological toxicity was more frequent in the valganciclovir group (19). Conclusions regarding the optimal dose of valganciclovir could not be reached (19).

Close surveillance for CMV viremia using quantitative assays is an approach that allows both early detection of viral load and prompt intervention in order to avoid CMV infections. The initiation of 900 mg valganciclovir twice daily when the threshold of 400 copies/mL of CMV load is exceeded was inferior to prophylactic treatment with 450 mg of valganciclovir twice daily in CMV episodes prevention but had similar effects on rejection episodes and allograft and overall survival on the long run (20). Close monitoring of CMV replication is to be considered during the direct period post valganciclovir prophylaxis discontinuation in high-risk patients as both myelotoxicity minimization by avoiding prolonged exposure to valganciclovir and proper treatment are feasible (18, 21).

Intravenous Immunoglobulin

IVIG preparations are produced via the extraction of immunoglobulins from healthy individuals (22). Currently IVIG is used for the management of numerous conditions which include immunodeficiency syndromes, inflammatory systemic diseases (SLE included) and hematologic conditions such as idiopathic thrombocytopenic purpura (23). A big effort has been made to describe the underlying mechanism of action for this multi-purpose therapeutic option which is characterized by both pro- and anti-inflammatory action. In brief, low dose IVIG seems to exert its pro-inflammatory action through complement and innate immunity cell activation achieved by Fc-IgG fragment binding with its receptor (FcγR) on recipient's cells giving rise to the expression of stimulatory signals and enhancing cell mediated toxicity (23, 24). While the aforementioned mechanism is thought to summarize the benefit in patients with IS, IVIG in higher doses acts as anti-inflammatory agent. Among the proposed characteristics is the scavenging of anaphylatoxins that leads to complement inhibition, the competitive action against autoantibodies and immunocomplexes regarding ligation on FcγRs decreasing granulocytes' activation, the enhancement of the inhibitory FcγRIIB expression inhibiting macrophages and modifying cytokine profile (22).

The applicability of IVIG in renal transplantation so far, involves desensitization protocols before transplantation as in ABO blood type incompatibility or in highly HLA sensitized patients, administered as monotherapy or combined with rituximab and/or plasmapheresis and finally as substitution in secondarily induced agammaglobulinemia in the context of recurrent infections (BK virus, parvovirus B19, CMV) that may cause intrinsic allograft nephropathy if left untreated (22). Although not validated with controlled studies, another indication for IVIG in kidney transplantation is antibody mediated rejection (AMR) (22) with good short-term effectiveness (25). IVIG seems to be well-tolerated and most postulated side effects concern infusion related symptoms or rare

TABLE 1 | Immunoglobulin levels timeline.

	(Normal range)	On CAPD initiation	18 months later-During CMV infection	Measurements while infection free (4year period)		2-months after IVIG initiation	6-months after IVIG initiation/3-months post-Tx
IgG (mg/dl)	751–1560	406	129	<33	<33	424	1160
IgA (mg/dl)	82–453	89	54	<7	<7	<7	<7
IgM (mg/dl)	46–304	59	41	573	667	347	91
IgE (mg/dl)	5–165	16		<5	8		6

A gradual restoration of IgG levels was achieved along with normalization of IgM levels, after induction of IVIG treatment.
Ig, immunoglobulin; CAPD, continuous ambulatory peritoneal dialysis; CMV, cytomegalovirus; IVIG, immunoglobulin.

TABLE 2 | Patient's viral immunology profile changes in time.

		On CMV infection	1 month later	6 months later	4-year period on infection free workups		4 months after IVIG initiation	7 months after IVIG initiation/4 months post-Tx
CMV IgM	>22 positive	(inconclusive)	133	100	101	64.8	67	33.9
CMV IgG	>14 positive	<5		<5	<5	5	82	116
PCR CMV (copies/ml)		157.000	1.200	0		0	0	0
EBV IgM	>1 positive	<10		<1	<1	<1	<1	<1
EBV IgG	>20 positive	11		<11	<1	<1	63.36	69.18
anti-HBs (mIU/ml)	< 10 negative			7.2	0.086	0.07	776.4	>1000
anti-HBc IgG	>1 positive			<1	<1	<1	<1	<1

Major remarks are the absent CMV IgGs after the resolution of CMV viremia and the seroconversion on both CMV IgGs and anti-HBs after intravenous IVIG treatment.
CMV, cytomegalovirus; PCR, polymerase chain reaction; EBV, Epstein Barr virus; HBs, Hepatitis B surface antigen; HBc, Hepatitis B core antigen; IVIG, intravenous immunoglobulin; Tx, transplantation.

incidents of acute kidney injury due to osmotic nephrosis mainly with saccharose-containing preparations (25).

The Case

Our patient, a young woman that reached ESKD 4 years after SLE diagnosis, remained on CAPD for 7 years. In the meantime, there were no flare-ups of her primary disease and no need for treatment step up apart from maintenance with low-dose prednisolone and hydroxychloroquine. The activity of SLE that declines in parallel with ESKD progression in time has already been described with gradual remission of lupus related manifestations and diminished serological activity (26). Nevertheless, the major concern about our patient were recurrent upper respiratory infections and the diagnosis of CMV infection. The case is further complicated by the subsequent severe hypogammaglobulinemia (**Table 1**) and patient's failure in acquiring immunity for CMV with undetectable CMV IgG levels (**Table 2**). On the other hand, there is a sustained positivity of low level CMV IgM antibodies with untraceable viral load after repeated PCR exams until present, which could be of minor importance as long as it is not accompanied by clinical and laboratory (i.e., leukopenia) manifestations or other indications of intrinsic disease.

Successful kidney transplantation is the best possible option for ESKD patients. The time dependent "burn-out" effect of autoimmunity (27), but mainly the experience gained from kidney transplantation in patients with lupus nephritis has improved outcomes and allograft survival (28). Available data regarding immunodeficiency syndromes and kidney transplantation are restricted in a small number of case reports. In such a case, a patient with primary immunodeficiency on maintenance IVIG proceeding to kidney transplantation had an uncomplicated postoperative course with direct allograft function and co-administration of immunosuppression consisting of basiliximab, tacrolimus (trough levels 3.9 ng/mL), mycophenolate mofetil and corticosteroids (29). This patient presented with allograft rejection after 2 and a half years, with de-novo donor specific antigens due to non-compliance with medication (29). In another case, a 15-year-old transplant patient treated with antithymocyte globulin, azathioprine, cyclosporine A and methylprednisolone, was diagnosed with IS 2 years post transplantation after multiple episodes of acute sinusitis and oral candidiasis, which in several instances were accompanied by acute allograft rejection episodes. However, after immunoglobulin substitution with IVIG the patient remained infection free with stable renal function (30). In a single-center case series, 3 patients with ESKD and hypo-gammaglobulinemia

on IVIG treatment, received a kidney transplant with minor complications. Among them, a young woman with SLE, 6 months after transplantation suffered from extensive herpetic skin infection and oral and vaginal candidiasis that apart from antiviral and antifungal treatment was managed with minimization of her immunosuppression (31). An important aspect in this regard is the potential immunomodulatory effect of the administered IVIG and the consequent potential protection against antibody mediated rejection episodes. In this light, an additional question arises about the safe reduction of standard immunosuppressive therapy under the protective permanent IVIG administration, with the ultimate goal of reducing calcineurin inhibitor (CNI) toxicity on the one hand and preventing episodes of infection on the other.

Appropriate management of our patient's acquired immunodeficiency was initiated with the administration of IVIG 500 mg/kg every 2 weeks targeting at IgG levels > 500 mg/dl. About 4 months later a change in serologic profile was observed with positive IgG for several viruses, as shown in **Table 2**, and among them IgGs against surface HBV antigen. It is worth noting that the patient was a non-responder after the completion of HBV vaccination. Similar observations have already been reported in two pediatric patients (32) and in a Canadian cohort of 11 patients with ITP, 3 of whom had a seroconversion with positive anti-core IgG for HBV, post IVIG administration (32, 33). Retesting in the following weeks, after pausing IVIG revealed a return to former status, concluding that the passive transfer of pre-formed immunoglobulins was the etiology of the case (33). Overall, substitution with IVIG in solid organ transplant recipients with hypoglobulinemia seems to protect against severe infections (34) including CMV infection and lowers allograft rejection incidence (35). The restoration of IgG levels with IVIG in patients with multiple infections post-transplantation prevented this deleterious continuality (36) and improved overall survival (37).

The half-life of IVIG varies from 3 to 4 weeks, depending on the formulation administered (38). However, the question about the effectiveness and functionality of transferred immunoglobulins and whether they are indicative of immunity as reflected on the laboratory measurements still remains. The significance of this remark is further underlined when it comes to our patient's history of cytomegalovirus infection, the absence of CMV IgG antibodies even after the resolution of viremia and on the other hand the seroconversion achieved after IVIG therapy. Queries for the clinical team are still pending: the measured IgGs provide protection against CMV infection? Prophylactic treatment with valganciclovir should be prolonged after 3 or even 6 months of treatment even if there are no supporting data available (18, 21)?

CONCLUSIONS

SLE is a disease with multiple comorbidities and ESKD may be reached not long after the diagnosis despite all efforts for tailor made immunosuppression. Even if renal replacement therapy ensures patient's survival and a good quality of life, a successful kidney transplantation remains the gold standard of treatment. Nevertheless, the management of a transplant patient can be complex when immunity dysregulations coexist, as the need for B-cell products substitution, that is, gammaglobulins via IVIG administration on one hand and T-lymphocytes suppression in order to avoid allograft rejection on the other may be simultaneously necessary. The confined data regarding kidney transplantation in patients with IS should not be discouraging as long as there is an integrated medical team and close monitoring so that early intervention according to patient's needs is undertaken.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article. A written informed consent was obtained by the patient prior to writing this article.

AUTHOR CONTRIBUTIONS

PP wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

REFERENCES

1. Tektonidou MG, Dasgupta A, Ward MM. Risk of end-stage renal disease in patients with lupus nephritis, 1971-2015: a systematic review and Bayesian meta-analysis. *Arthritis Rheumatol.* (2016) 68:1432–41. doi: 10.1002/art.39594
2. Feldman CH, Marty FM, Winkelmayer WC, Guan H, Franklin JM, Solomon DH, et al. Comparative rates of serious infections among patients with systemic lupus erythematosus receiving immunosuppressive medications. *Arthritis Rheumatol.* (2017) 69:387–97. doi: 10.1002/art.39849
3. Hsu CY, Lin MS, Su YJ, Cheng TT, Lin YS, Chen YC, et al. Cumulative immunosuppressant exposure is associated with diversified cancer risk among 14 832 patients with systemic lupus erythematosus: a nested case-control study. *Rheumatology.* (2017) 56:620–8. doi: 10.1093/rheumatology/kew457
4. Sawada T, Fujimori D, Yamamoto Y. Systemic lupus erythematosus and immunodeficiency. *Immunol Med.* (2019) 42:1–9. doi: 10.1080/25785826.2019.1628466
5. Errante PR, Perazzio SF, Frazão JB, da Silva NP, Andrade LEC. Primary immunodeficiency association with systemic lupus erythematosus: review of literature and lessons learned by the Rheumatology Division of a tertiary university hospital at São Paulo, Brazil. *Revista Brasileira de Reumatologia.* (2016) 56:58–68. doi: 10.1016/j.rbre.2015.07.006

6. Schmidt RE, Grimbacher B, Witte T. Autoimmunity and primary immunodeficiency: Two sides of the same coin? *Nat Rev Rheumatol.* (2018) 14:7–18. doi: 10.1038/nrrheum.2017.198
7. Javier Chinen, William T. Shearer. Secondary immunodeficiencies, including HIV infection Javier. *Physiol Behav.* (2016) 176:139–48. doi: 10.1016/j.jaci.2009.08.040
8. Bonilla FA, Barlan I, Chapel H, Costa-Carvalho BT, Cunningham-Rundles C, de la Morena MT, et al. International consensus document (ICON): common variable immunodeficiency disorders. *J Allergy Clin Immunol.* (2016) 4:38–59. doi: 10.1016/j.jaip.2015.07.025
9. Smith T, Cunningham-Rundles C. Primary B-cell immunodeficiencies. *Human Immunol.* (2019) 80:351–62. doi: 10.1016/j.humimm.2018.10.015
10. Raje N, Dinakar C. Overview of Immunodeficiency DisoCivelek, Mete Lusis, Aldons J Genetics, Molecular Angeles, Losrders. *Immunol Allergy Clin North Am.* (2015) 35:599–623. doi: 10.1016/j.iac.2015.07.001
11. Na IK, Buckland M, Agostini C, Edgar JDM, Friman V, Michallet M, et al. Current clinical practice and challenges in the management of secondary immunodeficiency in hematological malignancies. *Eur J Haematol.* (2019) 102:447–56. doi: 10.1111/ejh.13223
12. Yatim KM, Lakkis FG. A brief journey through the immune system. *Clin J Am Soc Nephrol.* (2015) 10:1274–81. doi: 10.2215/CJN.10031014
13. Patel SY, Carbone J, Jolles S. The expanding field of secondary antibody deficiency: causes, diagnosis, and management. *Front Immunol.* (2019) 10:33. doi: 10.3389/fimmu.2019.00033
14. Weening JJ, D'Agati VD, Schwartz MM, Seshan S v, Alpers CE, Appel GB, et al. The classification of glomerulonephritis in systemic lupus erythematosus revisited. *Kidney Int.* (2004) 65:521–30. doi: 10.1111/j.1523-1755.2004.00443.x
15. Fine RN. Tolerance in solid-organ transplant. *Exp Clin Transplant.* (2016) 14(Suppl 3):1–5.
16. Lowance D, Neumayer HH, Legendre CM, Squifflet JP, Kovarik J, Brennan PJ, et al. Valacyclovir for the prevention of cytomegalovirus disease after renal transplantation. International Valacyclovir Cytomegalovirus Prophylaxis Transplantation Study Group. *N Engl J Med.* (1999) 340:1462–70. doi: 10.1056/NEJM199905133401903
17. Večerić-Haler Ž, Bizjak B, Romozi K, Arnol M. Expanded valganciclovir prophylaxis in kidney transplant recipients is associated with lower incidence of cytomegalovirus infection. *Clin Nephrol.* (2017) 88:S126–30. doi: 10.5414/CNP88FX27
18. Kotton CN, Kumar D, Caliendo AM, Huprikar S, Chou S, Danziger-Isakov L, et al. The third international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. *Transplantation.* (2018) 102:900–31. doi: 10.1097/TP.0000000000002191
19. Florescu DF, Qiu F, Schmidt CM, Kalil AC. A direct and indirect comparison meta-analysis on the efficacy of cytomegalovirus preventive strategies in solid organ transplant. *Clin Infect Dis.* (2014) 58:785–803. doi: 10.1093/cid/cit945
20. Witzke O, Nitschke M, Bartels M, Wolters H, Wolf G, Reinke P, et al. Valganciclovir prophylaxis versus preemptive therapy in cytomegalovirus-positive renal allograft recipients: long-term results after 7 years of a randomized clinical trial. *Transplantation.* (2018) 102:876–82. doi: 10.1097/TP.0000000000002024
21. Razonable RR, Humar A. Cytomegalovirus in solid organ transplant recipients-Guidelines of the American Society of Transplantation Infectious Diseases Community of Practice. *Clin Transplant.* (2019) 33:e13512. doi: 10.1111/ctr.13512
22. Perez EE, Orange JS, Bonilla F, Chinen J, Chinn IK, Dorsey M, et al. Update on the use of immunoglobulin in human disease: a review of evidence. *J Allergy Clin Immunol.* (2017) 139:S1–46. doi: 10.1016/j.jaci.2016.09.023
23. Durandy A, Kaveri SV, Kuijpers TW, Basta M, Miescher S, Ravetch JV, et al. Intravenous immunoglobulins-understanding properties and mechanisms. *Clin Exp Immunol.* (2009) 158(Suppl. 1):2–13. doi: 10.1111/j.1365-2249.2009.04022.x
24. Nimmerjahn F, Ravetch J v. Anti-inflammatory actions of intravenous immunoglobulin. *Ann Rev Immunol.* (2008) 26:513–33. doi: 10.1146/annurev.immunol.26.021607.090232
25. Jordan SC, Toyoda M, Kahwaji J, Vo AA. Clinical aspects of intravenous immunoglobulin use in solid organ transplant recipients. *Am J Transplant.* (2011) 11:196–202. doi: 10.1111/j.1600-6143.2010.03400.x
26. Mojcik CF, Klippel JH. End-stage renal disease and systemic lupus erythematosus. *Am J Med.* (1996) 101:100–7. doi: 10.1016/s0002-9343(96)00074-5
27. Fries JF, Powers R, Kempson RL. Late-stage lupus nephropathy. *J Rheumatol.* (1974) 1:166–75.
28. Contreras G, Mattiazzi A, Guerra G, Ortega LM, Tozman EC, Li H, et al. Recurrence of lupus nephritis after kidney transplantation. *J Am Soc Nephrol.* (2010) 21:1200–7. doi: 10.1681/ASN.2009101093
29. al Nimri O, Rajput A, Martinez E, Fahrenholz JM, Paueksakon P, Langone A, et al. Acute rejection of a kidney transplant in a patient with common variable immunodeficiency: a case report. *Transplant Proc.* (2017) 49:380–5. doi: 10.1016/j.transproceed.2016.12.014
30. Hogan MB, Wilson NW, Muchant DG. Renal transplantation in a patient with common variable immunodeficiency. *Am J Kidney Dis.* (1999) 33:6–9. doi: 10.1016/S0272-6386(99)70174-5
31. Lund KP, Bruunsgaard H, Marquart HV, Sørensen SS. Case Report: renal transplantation in patients with pre-existing hypogammaglobulinemia. *Scand J Immunol.* (2017) 86:113–7. doi: 10.1111/sji.12570
32. Ilboudo CM, Guest EM, Ferguson AM, Garg U, Jackson MA. Misleading hepatitis B testing in the setting of intravenous immunoglobulin. *F1000Res.* (2013) 2:1–7. doi: 10.12688/f1000research.2-249.v1
33. Arnold DM, Crowther MA, Meyer RM, Carruthers J, Ditomasso J, Heddle NM, et al. Misleading hepatitis B test results due to intravenous immunoglobulin administration: implications for a clinical trial of rituximab in immune thrombocytopenia. *Transfusion.* (2010) 50:2577–81. doi: 10.1111/j.1537-2995.2010.02766.x
34. Sarmiento E, Diez P, Arraya M, Jaramillo M, Calahorra L, Fernandez-Yañez J, et al. Early intravenous immunoglobulin replacement in hypogammaglobulinemic heart transplant recipients: results of a clinical trial. *Transplant Infect Dis.* (2016) 18:832–43. doi: 10.1111/tid.12610
35. Sarmiento E, Fernàndez-Yáñez J, Muñoz P, Palomo J, Rodríguez-Molina JJ, Bermejo J, et al. Hypogammaglobulinemia after heart transplantation: use of intravenous immunoglobulin replacement therapy in relapsing CMV disease. *Int Immunopharmacol.* (2005) 5:97–101. doi: 10.1016/j.intimp.2004.09.006
36. Carbone J, Sarmiento E, del Pozo N, Rodriguez-Molina JJ, Navarro J, Fernandez-Yañez J, et al. Restoration of humoral immunity after intravenous immunoglobulin replacement therapy in heart recipients with post-transplant antibody deficiency and severe infections. *Clin Transplant.* (2012) 26:E277-83. doi: 10.1111/j.1399-0012.2012.01653.x
37. Carbone J, Sarmiento E, Palomo J, Fernandez-Yañez J, Muñoz P, Bouza E, et al. The potential impact of substitutive therapy with intravenous immunoglobulin on the outcome of heart transplant recipients with infections. *Transplant Proc.* (2007) 39:2385–8. doi: 10.1016/j.transproceed.2007.06.050
38. Peart W. Immune globulin subcutaneous (human) vivaglobin: for the treatment of primary immunodeficiency diseases. *P and T.* (2009) 34:1–21.
39. Tedla FM, Roche-Recinos A, Brar A. Intravenous immunoglobulin in kidney transplantation. *Curr Opin Organ Transplant.* (2015) 20:630–7. doi: 10.1097/MOT.0000000000000250

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

REVIEW
published: 19 March 2021
doi: 10.3389/fmed.2021.661203

Microbiome, Immunosenescence, and Chronic Kidney Disease

Elisavet Stavropoulou[1,2], Konstantia Kantartzi[3]*, Christina Tsigalou[4], Konstantina Aftzoglou[5], Chrysa Voidarou[6], Theocharis Konstantinidis[7], Mariana Carmen Chifiriuc[8], Elias Thodis[3] and Eugenia Bezirtzoglou[9]*

[1] CHUV (Centre Hospitalier Universitaire Vaudois), Rue du Bugnon, Lausanne, Switzerland, [2] Department of Infectious Diseases, Central Institute, Valais Hospital, Sion, Switzerland, [3] Nephrology Clinic, Department of Medicine, Democritus University of Thrace, Alexandroupoli, Greece, [4] Laboratory of Microbiology, Department of Medicine, Democritus University of Thrace, Alexandroupoli, Greece, [5] Medical School, Comenius University, Bratislava, Slovakia, [6] Department of Public Health P.U., Arta, Greece, [7] Department of Medicine, Democritus University of Thrace, Alexandroupoli, Greece, [8] Laboratory of Microbiology, Faculty of Biology, The Research Institute of the University of Bucharest (ICUB), University of Bucharest, Bucharest, Romania, [9] Laboratory of Hygiene and Environmental Protection, Department of Medicine, Democritus University of Thrace, Alexandroupoli, Greece

The gut microbiome is known as an important predictive tool for perceiving characteristic shifts in disease states. Multiple renal diseases and pathologies seem to be associated with gut dysbiosis which directly affects host homeostasis. The gastrointestinal-kidney dialogue confers interesting information about the pathogenesis of multiple kidney diseases. Moreover, aging is followed by specific shifts in the human microbiome, and gradual elimination of physiological functions predisposes the microbiome to inflammaging, sarcopenia, and disease. Aging is characterized by a microbiota with an abundance of disease-associated pathobionts. Multiple factors such as the immune system, environment, medication, diet, and genetic endowment are involved in determining the age of the microbiome in health and disease. Our present review promotes recently acquired knowledge and is expected to inspire researchers to advance studies and investigations on the involved pathways of the gut microbiota and kidney axis.

Keywords: gut, kidney, gut-kidney axis, microbiome, ageing, immunosenescence, chronic kidney disease, microbiota

OPEN ACCESS

Edited by:
Maria J. Stangou, Aristotle University of Thessaloniki, Greece

Reviewed by:
Evangelos Papachristou, University of Patras, Greece
Erasmia Sampani, Hippokration General Hospital Thessaloniki Greece, Greece

****Correspondence:***
Elisavet Stavropoulou
elisabeth.stavropoulou@gmail.com
Konstantia Kantartzi
kkantart@med.duth.gr

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *30 January 2021*
Accepted: *23 February 2021*
Published: *19 March 2021*

Citation:
Stavropoulou E, Kantartzi K, Tsigalou C, Aftzoglou K, Voidarou C, Konstantinidis T, Chifiriuc MC, Thodis E and Bezirtzoglou E (2021) Microbiome, Immunosenescence, and Chronic Kidney Disease. Front. Med. 8:661203. doi: 10.3389/fmed.2021.661203

INTRODUCING THE AGING MICROBIOME

The human newborn is devoid of bacteria at birth (1). Bacteria colonizing the sterile newborn either come from the hospital environment and staff as in the case of caesarian section or from normal maternal vaginal microflora (1). The establishment and progression of the human microflora is attributed to the influence of multiple epigenetic mechanisms (2). Personal habits and behavior, stress, hormones, antibiotics, vaccination, and infections (1) seem to be involved. However, nutrition remains the ultimate factor that can sway newborn development processes regulating epigenetic mechanisms during pregnancy and early life (1, 2). The importance of food intake variations is stated by a plethora of publications (1–4). Studies comparing children in rural Africa and Europe reported important variations in microbial populations due to eating habits (5). African children were colonized by more bacteria belonging to *Actinobacteria* phylum and *Bacteroidetes* than *Firmicutes* compared to European children group who carried more *Firmicutes and Proteobacteria.*

The importance of early life colonization is understood (1). The presence of beneficial bacteria such as *Lactobacilli* and *Bifidobacteria* protect against disease (6). The "Hygiene Hypothesis" was

advanced to explain atopic disorders after immune dysregulation (7). Human microbiota evolve in parallel with the immune system supporting a bidirectional relationship resulting in normal immune development (8).

Nowadays, the term "microflora" is used less frequently in favor of the term "microbiota" as microbial genomes are also involved. The term was first used by the Nobel Laureate Joshua Lederberg (9).

Bacterial communities are involved in complex inter-communication and network models of unique microbiomes. In this vein, characterization of the different microbial communities in health and disease status was achieved due to new technological involvements and particularly 16S rRNA sequencing. This methodology permits the identification of complex microbial populations in the human body (10). Additionally, metagenomics Whole Genome Shotgun (WGS) sequencing has allowed for the identification of involved functions in relation to our microbiome (10, 11). It seems to be less crucial to confirm "who is there" than "what are they doing." The *Human Microbiome Project* (USA) (12) as well as *the metaHIT Consortium* (Europe) (13) have shed light on the characterization of major healthy human sites in order to compare them with shifts occurring in disease states (14).

In developed countries, during the last century, improvements in healthcare have led to a population of higher age and life expectancy has risen (15). With the recognition of an aging population (16), geriatric research has gained the interest of multiple society sectors including topics such as social, work, and economic impact and nutrition and health issues.

It is known that frail and elderly people encounter more infections than younger people (17). Infections in elderly subjects are often complicated due to multi-morbidity (17), hormonal shifting, increased production of pro-inflammatory cytokines and chemokines, and abnormalities of the telomeres which finally could cause a dysfunction of the immune system called immunosenescence and malnutrition.

The impact of aging upon the intestinal microbiota is associated with a decrease in the anaerobic population (18, 19), specifically the *Bifidobacterial* population (20, 21), while an increase in E*nterobacterial* population has been reported (19, 21).

Age-related sequential changes were reported in the human microbiota (22) by 16SrRNA methodologies. *Actinobacteria* phylum (mainly *Bifidobacteria*) was decreased with age and after weaning (22), while *Firmicutes* (mainly *Clostridium* cluster XIVa and *Faecalibacterium* 57 *prausnitzii*) were more frequent in older children but at lower levels (23). Finally, *Bacteroidetes* and *Proteobacteria* were found in human recipients over 70 years old (22). Taking it one step further, in analysis focusing on bacterial co-abundance groups (CAGs) as defined by Kendall, correlations between genera showed that several transition types of microbiota were enriched in the adult population (22). Relative abundance of genera was registered in elderly-associated CAGs compared to infant- and adult-associated CAGs (22). Linkage clustering based on the abundance of genera indicated five age clusters with median ages 3, 33, 42, 77, and 94 years old (22). However, when clustering was based on the proportion of transporters evaluated by phylogenetic analysis of the bacterial communities by reconstruction of unobserved states (PICRUSt), the human recipients were classified into two age groups; the adult-enriched and the infant/elderly-enriched clusters (22).

IMMUNOLOGICAL PATHWAYS IN KIDNEY DISEASE

It is known that the intestine possesses dual functions, firstly a role in nutrient absorption and also a function in the synthesis of substances such as amino acids, vitamins, and short chain fatty acids (SCFAs) (24). SCFAs exert beneficial effects, confer energy to epithelial cells, and engage in a potent role in the immunomodulation and barrier effect against pathogenic invaders (24). Particularly, they hold two basic signaling functions; the activation of G-protein-coupled receptors (GPCRs) and the inhibition of histone deacetylases (HDACs) (25). GPCRs are receptors of SCFAs which participate in metabolism, inflammation, and disease processes (25). Still, SCFAs are activated in the free fatty acid receptor-2 and−3 (FFAR2 and FFAR3) found in numerous human body sites (26). Additionally, SCFAs upset the physiology of the intestinal epithelial cells by inhibiting histone deacetylases (HDACs) resulting in chromatin remodeling and changes in transcription processes (27). Finally, HDACs seem to possess an anti-proliferative and anti-inflammatory action either *in vitro* or *in vivo* in developed models of inflammation (27).

In this vein, the intestinal microbiota *via* the intestinal barrier seem to adjust homeostasis and functions of both innate and adaptive immunity locally and systemically (28). However, when the intestinal barrier is breached, a situation called "leaky gut," the gut bacteria and their toxins are able to infiltrate the intestinal mucosa and then through the blood stream circulate to different tissues and organs (1, 29). Moreover, activated immune cells penetrate the kidney and generate pro-and anti-inflammatory reactions and regulatory signals in order to induce a neutrophils response (30). Neutrophils together with macrophages are induced as part of the first line response in innate immunity against pathogens (31) and kidney disease (32).

Impairment of the macrophages' phagocytic ability has a negative effect on kidney function leading to chronic inflammation (31). Chronic systemic inflammation can be appraised using the neutrophil-to-lymphocyte ratio (NLR) which is associated with the risk of ESRD with stage 4 CKD. NLR could be a prognostic marker for cardiovascular risk and mortality in patients with CKD 3-5 and hemodialysis-peritoneal dialysis patients, respectively (33, 34).

To this end, the role of pattern recognition receptors (PRRs), and especially TLRs (toll like receptors) which are membrane glycoproteins, during inflammation processes is stated (31). TLRs are found in renal cells and activate mitogen-activated protein kinases, nuclear factor-κB, and activator protein-1 toward a pro-inflammatory status (35, 36). The importance of a dialogue between the acquired immune system and the innate system is understood (37, 38) through the production of cytokines.

Renal tubular epithelial cells participate in immunity processes by producing chemokines, cytokines, and

antimicrobial substances (32). In their turn, cytokines participate in the immunological response by promoting the synthesis of acute phase proteins and tissue proteolysis and lipolysis. Moreover, they interact with T lymphocytes to generate the acquired immune response (39). A cell-mediated response to the antigen will take place and T lymphocytes will similarly produce cytokines in order to regulate the activity of immunocompetent cells and induce antibody production (40). Injured renal tubular epithelial cells dedifferentiate to achieve refit and thus they incite inflammation by recruiting myofibroblasts. In this way, tubular epithelial cell loss stimulates residual renal hypertrophy. Thus, the hypertrophied nephron is unable to cope with the increase in tubular transport as it overwhelms its energy-generating capacity, and anaerobic metabolism, acidosis, and hypoxia occurs (41, 42).

Renal tubular epithelial cells present a crucial role in inflammation, positively or negatively regulating T cell responses in an alternative way, as they express co-stimulators of T cells (ICOS-L) and B7-H1 molecules (43). Yet, macrophages, dendritic cells (DCs), and T regulatory cells (Tregs) induce an adaptive immune response and DC activation promotes the production of proinflammatory cytokines such as IL-12 and IL-6 (30). Clearly, DCs trigger the differentiation of naïve $CD4^+$ T cells into regulatory T (Treg) cells and the maturation of B cells into IgA-secreting ones (30, 44). The role of Treg cells in renal disease seems to be crucial in protecting against inflammation processes and amplifying homeostasis by boosting microbiota (45). In support, T helper 17 (Th17) cells are activated inducing the production of pro-inflammatory interleukin-17 (IL-17) (46). To this end, nuclear factor-κB (NF-κB) is released by the renal tubular epithelial cells regulating pro-inflammatory response (47).

Furthermore, innate lymphoid cells (ILCs) tamper with pro-inflammatory cytokines IL-1β, IL-12, IL-23, IL-22, and IFNγ production (44, 48). It was also found that the aryl hydrocarbon receptor of IL-22 in innate lymphoid cell response (ILC3) suppresses inflammatory Th17 cell responses and regulates Treg-mediated gut homeostasis (49). So then, the suppression of Th17 cells in the intestine confers positively to the translocation and activation in the kidney (44). Gut expressed Th cells can be activated in the kidney through the CCL20/CCR6 axis (50).

The intestinal microbiota cooperate by means of microbial associated molecular patterns (MAMPs) or SCFAs as previously discussed to temper inflammation in the kidney (51). It is of note that by the aid of RT-PCR, four receptors (GPR41, GPR43, Olfr78, and GPR109a) expressed in the kidney are linked to particular pathologies (52) (**Figure 1**).

Without any doubt, important physiological changes occur in the kidney as a result of immunoactivation. Immune cells and inflammatory proteins contribute to the pathogenesis of kidney diseases (53). Finally, it is worth noting the importance of the dialogue between the kidney and gut, the so-called gut-kidney axis in health and disease (54).

Actually, in spite of the technological advancements in peritoneal dialysis (PD) and hemodialysis (HD) procedures, the mortality in ESRD remains high (55) as cardiovascular disease and infections occurred in these patients. It seems that both complications are associated with immunological shifts in ESRD such as uremia (55). Uremia is characterized by immune dysfunction and immunosuppression leading to multiple infections. The accumulation of pro-inflammatory cytokines takes place as a result of dropped renal elimination capacity, oxidative stress, and the accumulation of uremic toxins. Moreover, immunoactivation results in inflammation and cardiovascular disease. Immune dysfunction in uremia is linked to both innate and adaptive immunity (55). Yet, adaptive immunity is altered in ESRD patients. It seems to be caused by uremia *per se* and chronic renal failure. T cell proliferation is mitigated in an uremic environment. T helper lymphocytes (Th) have an impact on the immune response. Th1 cells activate macrophages, while Th2 cells promote humoral immunity (56). Interestingly, the maturation of Th cells in hemodialysis patients (HD) does occur, these subjects showed increased Th1 concentrations and an increased Th1/Th2 ratio (57). Studies state that ratio increase in HD is associated with the elevated production of IL-12 which effects T lymphocytes. This leads to an increase in IFN-γ and a decrease in IL-4, promoting their differentiation in Th1 cells (55). Yet, B cell lymphopenia is apparent due to apoptosis, despite the production of IgM and IgA in normal levels in dialysis patients (58). Following initiation of renal replacement therapy in HD or peritoneal dialysis (CAPD) subjects, the immunological status of patients was appraised (59). The percentage of CD4+CD28 null and CD8+CD28 null cells was found increased in ESRD patients. Therefore, CD4+CD28 null cells correlated with CRP and serum albumin levels while important differences in items of CD4+CD28 null and CD8+CD28 null cells were found in patients with cardiovascular disease. Shifts in the population of CD4+CD28 null cells was found following 6 months of dialysis. However, these changes showed significant differences between HD and CAPD patients (59), T cells subtypes are affected by CKD and a chronic inflammation disease is installed. This turmoil is enhanced in HD patients but alleviated in CAPD patients (59).

The intestinal microbiome of HD patients showed an increase in *Proteobacteria, Actinobacteria*, and *Firmicutes* with preponderance of the *subphylum Clostridia,* while a decrease in the *taxa Firmicutes* and *Actinobacteria* is found in CAPD patients (60). It is known that there is an interplay between the kidney and gut, called the gut-kidney axis (54, 61). Renal transplantation incites changes in the gut microbiota (62). Yet, hormones, environment, genetics, epigenetics, and pharmacogenetics seem to impact kidney allograft receivers (62).

Gut microbiota could incite antigen-presenting cells (APCs) and initiate immune response and alloimmune reactivity, as is the case in allogeneic bone marrow transplantation (HSCT) (63). However, when allograft recipients are submitted to gut decontamination, acute graft vs. host disease declines (64). A considerable shift in microbiota was found 1 month after transplantation. It is of note that patients hosting *Faecalibacterium prausnitzii* in their microbiota need higher tacrolimus therapeutic doses (65).

Researchers found that gut-associated lymphoid tissue (GALT) plays a key role in the evolvement of immunoglobulin A (IgA) nephropathy (IgAN) (66).

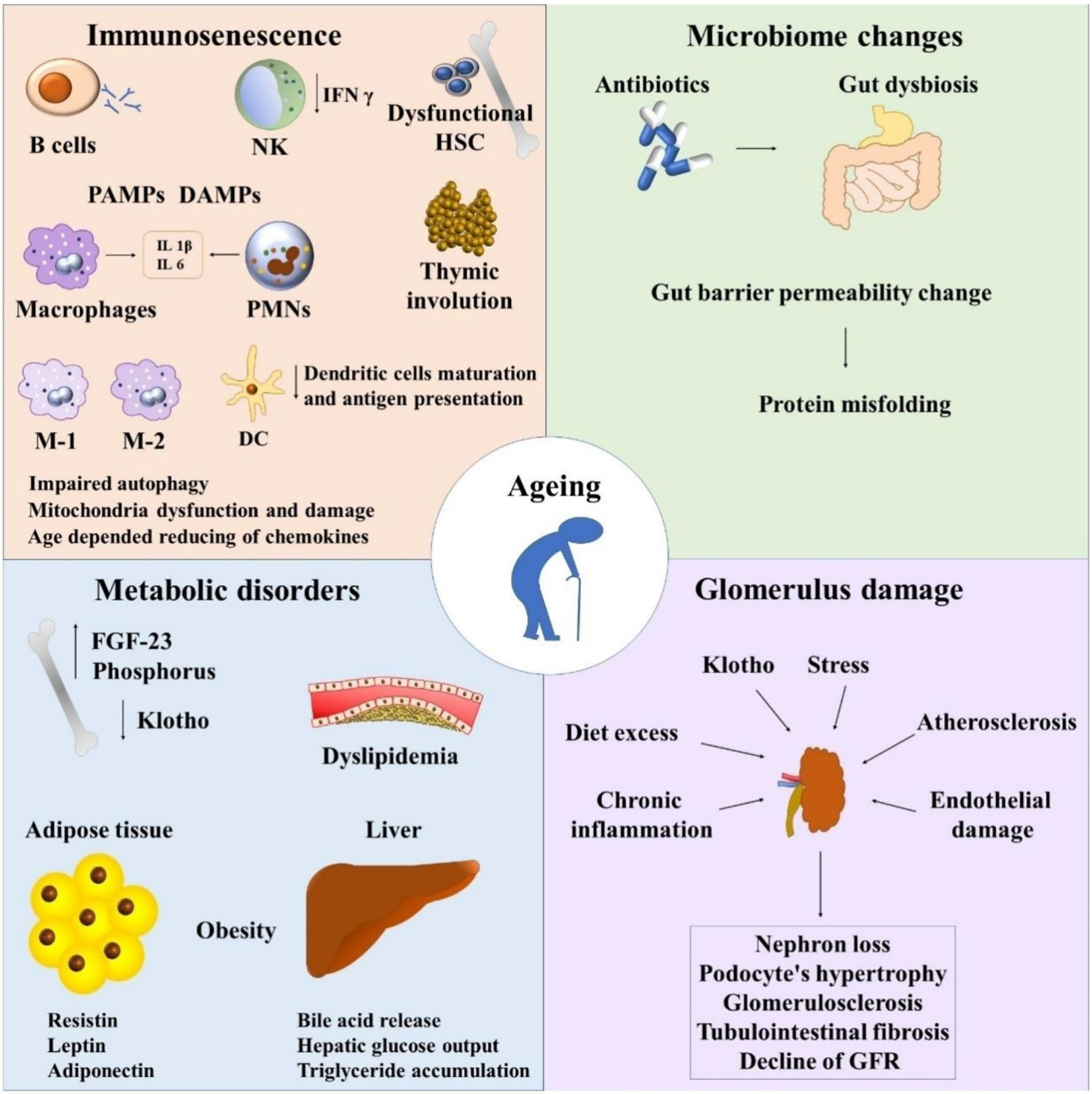


FIGURE 1 | Immunosenescence and chronic kidney disease. NK, natural killers; HSC, hematopoietic stem cells; PAMPs, pathogen-associated molecular pattern molecules; DAMPs, damage-associated molecular pattern molecules; PMNs, polymorphonuclear leukocytes; FGF-23, fibroblast growth factor 23; GFR, glomerular filtration rate.

Changes in gut microbiota and dysbiosis seem to be critical for immunoglobulin A nephropathy (IgAN) (54). In IgAN patients, an abundance of *Fusobacteria* is observed, while *Synergistetes* were decreased (67). Genome studies showed that IgAN and inflammatory bowel diseases are linked to the same loci (66). This observation involves a different clinical approach including a treatment option that focuses on subclinical intestinal inflammation or microbiota shifting (68).

Dysbiosis of the gut microbiota was also related to patients with idiopathic membranous nephrotic syndrome (INS) (69). *Fusobacteria, Proteobacteria,* and *Parabacteroides* are increased in INS patients, while *Firmicutes* dropped (69). At the genus level, ***Providencia*** and ***Myroides*** were found more frequently in INS patients (69). Yet, propionate acid and butyric acid are found in low concentrations in INS patients (69).

AGING AND SENESCENCE: TWO FACETS IN THE CONTEXT OF IMMUNITY

During aging, physiological and pathological changes emerge in contrast to senescence where mainly non-pathological changes occur. There is an impairment of multiple functions including the dermal, mucosal, and epithelial barrier and (50) the barrier effect (70). While most scientists have not found a quantitative variation in immunological cells with aging, B lymphocytes and T lymphocytes associated with adaptive immunity and natural killers cells, granulocytes, monocytes, and macrophages associated with the innate immunity were found in increased numbers (71, 72).

Yet, in elderly people, DCs showed a reduction in antigen presentation-function, impaired endocytosis, and reduced chemokine production (72). This reduced chemokine production leads to a decrease in cytotoxicity of the natural killer (NK) cells and a decreased killing capacity. While natural killer numbers do increase in healthy elderly people due to the enhanced activity of the markers cells CD56dim and CD57, function is impaired due to cytotoxicity. Therefore, an enhanced production of IL-4 and IL-10 and a decreased production of INF-γ in elderly subjects is observed.

The importance of natural killer (NK) cells in kidney infection and inflammation was previously discussed. Although, natural killer (NK) cells increase quantitatively in lymphatic organs, they showed a low proliferative capacity in the peripheral blood (73). Neutrophils make up 50–70% of human white blood cells and they play an essential role in the innate immune system. They remain stable in the peripheral blood and the bone marrow of the elderly, although they have low phagocytic and killing activity and are more vulnerable to apoptosis (74). Although monocytes also have stable quantitative levels in the peripheral blood of an aged subject, macrophage function is decreased (75, 76). Yet, a temperate phagocytosis, chemiotaxis, and oxidative activity is seen due to the release of ROS, as superoxide radical and hydrogen peroxide from different cells (76). Moreover, the antigen-presenting capacity is lower. Thus, infection occurring in the elderly will be long lasting and it is likely to develop into a chronic inflammation state more frequently. Similarly, the same profile was shown for dendritic cells (DCs) (77). Moreover, in the frail elderly, an extensive reactivity against auto-antigens and an enhanced release of the pro-inflammatory cytokines TNF-a and IL-6 was registered (78). It is of note that these pro-inflammatory cytokines are used as predictive biomarkers for comorbidities and mortality (17).

Shifts observed in the immunological structure during chronological aging induce a "prolific milieu" for the development of a chronic inflammation state, so-called "*inflammaging.*"

Age-related modifications are more pronounced in the adaptive immune system.

Chronological aging lends itself to the decrease of naïve T cells and the accumulation of oligoclonal memory and cytotoxic T cells (79). Upon the end of the thymus involution process at around 50 years of age, a drop in T cell levels is marked and globally observed age-related shifts are more noticeable (80). The decrease of CD8+ cells was more profound compared to CD4+ cell levels (81).

Although, B lymphocytes present a stable profile in the peripheral blood, the numbers of mnemonic B cells is enhanced in order to offset the drop in naïve B cells in the elderly. In support of that observation, insufficient production of specific antibodies following vaccination with advanced aged was shown (82).

Recapitalizing, important shifts are shown in immune system cells during aging which lead to thymic involution, clonal exhaustion, and rupture (83) (**Figure 1**).

The term immunosenescence was coined by Roy Walford (84) when he published his hallmark book entitled "The Immunologic Theory of Aging" (85). The term denotes the aging-related dysfunction of the immune system (72) associated with higher infection possibility.

However, there is some scientific disputation in defining the term "immunosenescence" (72). Scientists report immunosenescence as a dysfunction of the global immune system called the "damage theory of aging," while others believe that only specific parameters are altered (72) entangling the telomere proliferation mechanisms (86). Telomeres seem to have a crucial role in aging via regulating cellular responses and DNA damage (87). Telomeres should "cap" chromosome ends to inhibit activation of DNA repair. As a result, apoptosis or cell senescence occurs when the number of "uncapped" telomeres accrues (87) due to shortening of each telomere length. This fact highlights the cessation of cellular proliferation which defines the aging status. Finally, a lack of telomeres is reported as an immunosenescence status (87). It is of note that amplified cancer cells have active telomerases and a stable telomere length and as a result they do not senescence and even when telomeres are linked to oncogenes, cells tend to immortalize (86–88).

Aging is linked to important shifts in gene expression. Overexpression of p16 and p21 gene inhibitors of the cellular cycle induce faster senescence (89). In this way, the induction of senescence induced by gene inhibitors may be a new therapeutic approach in the treatment of cancer (89).

CHRONIC KIDNEY DISEASE AND IMMUNOSENESCENCE

The term "chronic kidney disease (CKD)" reflects lasting damage to the kidneys that can aggravate over time. Chronic kidney disease (CKD) and end-stage renal disease (ESRD) are a dominant medical challenge in the 21st century (90), as more than 1.2 million people died from CKD in 2017 showing a considerable increase in global-age prevalence and mortality in the last 20 years (91). In Oceania, sub-Saharan Africa, and Latin America, the burden of CKD was much higher compared to the disease burden in other countries (91). Patients may develop complications such as hypertension, anemia, heart and blood vessel diseases, and nerve damage (90). Diabetes and

hypertension may cause CKD, susceptibility to infection, and other associated pathologies (90).

Early detection of the disease is important, as the disease develops and may lead to kidney impairment that necessities dialysis and finally kidney transplantation (90) to survive.

CKD is defined according to the level of glomerular filtration rate (GFR) into five gradual stages from asymptomatic stage 1 to the end-stage renal disease (ESRD) stage 5. The stages 3–5 show a glomerular filtration rate (GFR) below 60 ml/min per 1.73 m^2 for 3 months or more (90). Other pathological co-morbidities as well as additional markers of kidney damage, such as proteinuria or hematuria for 3 months or more are co-estimated (92). The disease seems to be more common in the elderly population considering chronological aging. CKD is characterized by senescence, and CKD and ESRD patients appear to be biologically older (93) due to their global malfunction status, when compared to a healthy population.

Kidney cellular shifts and immune cell dysfunction lead to immunosenescence and apoptosis as previously discussed. Moreover, important changes are registered in the kidney glomerular filtration barrier by loss of podocytes (94) which lead to renal impairment. Proteinuria and other lesions advance podocyte loss or induce death (75, 94).

The autophagy process has a central role in controlling homeostasis and adjacent involved mechanisms involved in glomerular disease and maintains podocytes homeostasis in aging (95, 96).

Tubulo-interstitial renal fibrosis is a condition of the aged kidney which is defined as a progressive detrimental connective tissue deposition on the kidney parenchyma leading to renal function damage (43, 97). The epithelial to mesenchymal transition (EMT) of tubular epithelial cells is converted to mesenchymal fibroblasts. Thus, fibroblasts proliferate to the adjacent interstitial parenchyma (97) (**Figure 1**).

As stated, advanced aging deteriorates the immune system, increases susceptibility to infection (98), and converges a low-grade activation of the inflammation system called inflammaging (79). Stimuli such as exposure to pathogens, cellular debris, nutrients, and the gut microbiota sustain inflammaging (53, 99).

The gut microbiota is the corner stone in inflammaging due to its capacity to produce inflammatory products and dialogue with other organs and systems (54). However, it is clear that the underlying aging mechanisms still need to be explained through this trajectory in order to gain a better understanding of this global dysregulation and provide more effective therapeutic approaches.

AUTHOR CONTRIBUTIONS

ES: conceptualization, writing, and editing. KK and CT: formal analysis and writing. CV and KA: resources and writing. TK: design and editing. MCC: writing and editing. EB and ET: supervision, original draft preparation, and editing. ET contribute to the reviewing and editing of the paper. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Bezirtzoglou E, Stavropoulou E. Immunology and probiotic impact of the newborn and young children intestinal microflora. *Anaerobe.* (2011) 17:369–74. doi: 10.1016/j.anaerobe.2011.03.010
2. Li Y. Epigenetic mechanisms link maternal diets and gut microbiome to obesity in the offspring. *Front Genet.* (2018) 9:342. doi: 10.3389/fgene.2018.00342
3. Michaelsen KF, World Health Organization. *Feeding and Nutrition of Infants and Young Children: Guidelines for the WHO European Region, With Emphasis on the Former Soviet Countries*. Copenhagen: WHO Regional office for Europe (2000). p. 288.
4. Bezirtzoglou E, Romond C. Effect of the feeding practices on the establishment of bacterial interactions in the intestine of the newborn delivered by cesarean section. *J Perinat Med.* (1990) 18:139–43.
5. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci USA.* (2010) 107:14691–6. doi: 10.1073/pnas.1005963107
6. Wall R, Ross RP, Ryan CA, Hussey S, Murphy B, Fitzgerald GF, et al. Role of gut microbiota in early infant development. *Clin Med Pediatr.* (2009) 3:45–54. doi: 10.4137/CMPed.S2008
7. Rautava S, Ruuskanen O, Ouwehand A, Salminen S, Isolauri E. The hygiene hypothesis of atopic disease–an extended version. *J Pediatr Gastroenterol Nutr.* (2004) 38:378–88. doi: 10.1097/00005176-200404000-00004
8. Stiemsma LT, Reynolds LA, Turvey SE, Finlay BB. The hygiene hypothesis: current perspectives and future therapies. *Immunotargets Ther.* (2015) 4:143–57. doi: 10.2147/ITT.S61528
9. Lederberg J, McCray AT. "Ome Sweet" Omics-A genealogical treasury of words. Sci Phila. (2001) 15:8.
10. Ranjan R, Rani A, Metwally A, McGee HS, Perkins DL. Analysis of the microbiome: advantages of whole genome shotgun versus 16S amplicon sequencing. *Biochem Biophys Res Commun.* (2016) 469:967–77. doi: 10.1016/j.bbrc.2015.12.083
11. Morgan XC, Huttenhower C. Human microbiome analysis. *PLOS Comp Biol.* (2012) 8:e1002808. doi: 10.1371/journal.pcbi.1002808
12. NIH Human Microbiome Project - Home. (2020). Available online at: https://hmpdacc.org/
13. MetaHIT Consortium (Metagenomics of the Human Intestinal Tract consortium) - *Wellcome Sanger Institute*. (2021). Available online at: https://www.sanger.ac.uk/resources/downloads/bacteria/metahit/
14. The Human Microbiome Project Consortium, Huttenhower C, Gevers, D. Structure, function and diversity of the healthy human microbiome. *Nature.* (2012) 486:207–214. doi: 10.1038/nature11234
15. European Commission. *Directorate-General for Economic and Financial Affairs. The 2012 Ageing Report : Underlying Assumptions and Projection Methodologies*. LT: Publications Office (2011). Available online at: https://data.europa.eu/doi/10.2765/15373
16. WHO publications. global_health.pdf. Available online at: https://www.who.int/ageing/publications/global_health.pdf
17. Marengoni A, Angleman S, Melis R, Mangialasche F, Karp A, Garmen A, et al. Aging with multimorbidity: a systematic review of the literature. *Ageing Res Rev.* (2011) 10:430–9. doi: 10.1016/j.arr.2011.03.003
18. Samedi L, Linton Charles A. Evaluation of technological and probiotic abilities of local lactic acid bacteria. *J Appl Environ Microbiol.* (2019) 7:9–19. doi: 10.12691/jaem-7-1-3
19. Hébuterne X. Gut changes attributed to ageing: effects on intestinal microflora. *Curr Opin Clin Nutr Metab Care.* (2003) 6:49–54. doi: 10.1097/00075197-200301000-00008

20. Ljungh A, Wadstrom T. Lactic acid bacteria as probiotics. *Curr Issues Intest Microbiol*. (2006) 7:73–89.
21. Stavropoulou E, Bezirtzoglou E. Human microbiota in aging and infection: a review. *Crit Rev Food Sci Nutr*. (2019) 59:537–45. doi: 10.1080/10408398.2017.1379469
22. Odamaki T, Kato K, Sugahara H, Hashikura N, Takahashi S, Xiao J, et al. Age-related changes in gut microbiota composition from newborn to centenarian: a cross-sectional study. *BMC Microbiol.* (2016) 16:90. doi: 10.1186/s12866-016-0708-5
23. Salazar N, Valdés-Varela L, González S, Gueimonde M, Reyes-Gavilán CG de Los. Nutrition and the gut microbiome in the elderly. *Gut Microbes.* (2017) 8:82–97. doi: 10.1080/19490976.2016.1256525
24. Louis P, Flint HJ. Formation of propionate and butyrate by the human colonic microbiota. *Environ Microbiol.* (2017) 19:29–41. doi: 10.1111/1462-2920.13589
25. Tan J, McKenzie C, Potamitis M, Thorburn AN, Mackay CR, Macia L. The role of short-chain fatty acids in health and disease. *Adv Immunol.* (2014) 121:91–119. doi: 10.1016/B978-0-12-800100-4.00003-9
26. Layden BT, Angueira AR, Brodsky M, Durai V, Lowe WL. Short chain fatty acids and their receptors: new metabolic targets. *Transl Res J Lab Clin Med.* (2013) 161:131–40. doi: 10.1016/j.trsl.2012.10.007
27. Schilderink R, Verseijden C, de Jonge WJ. Dietary Inhibitors of Histone Deacetylases in Intestinal Immunity and Homeostasis. *Front Immunol.* (2013) 4:226. doi: 10.3389/fimmu.2013.00226
28. Maynard CL, Elson CO, Hatton RD, Weaver CT. Reciprocal interactions of the intestinal microbiota and immune system. *Nature.* (2012) 489:231–41. doi: 10.1038/nature11551
29. Romond M-B, Colavizza M, Mullié C, Kalach N, Kremp O, Mielcarek C, et al. Does the intestinal bifidobacterial colonisation affect bacterial translocation? *Anaerobe.* (2008) 14:43–8. doi: 10.1016/j.anaerobe.2007.09.003
30. Dhana E, Ludwig-Portugall I, Kurts C. Role of immune cells in crystal-induced kidney fibrosis. *Matrix Biol J Int Soc Matrix Biol.* (2018) 68–69:280–92. doi: 10.1016/j.matbio.2017.11.013
31. Guiteras R, Flaquer M, Cruzado JM. Macrophage in chronic kidney disease. *Clin Kidney J.* (2016) 9:765–71. doi: 10.1093/ckj/sfw096
32. Tang PC-T, Zhang Y-Y, Chan MK-K, Lam WW-Y, Chung JY-F, Kang W, et al. The emerging role of innate immunity in chronic kidney diseases. *Int J Mol Sci.* (2020) 21:4018. doi: 10.3390/ijms21114018
33. C-STRIDE study group, Yuan Q, Wang J, Peng Z, Zhou Q, Xiao X, et al. Neutrophil-to-lymphocyte ratio and incident end-stage renal disease in Chinese patients with chronic kidney disease: results from the Chinese Cohort Study of Chronic Kidney Disease (C-STRIDE). *J Transl Med.* (2019) 17:86. doi: 10.1186/s12967-019-1808-4
34. An X, Mao H-P, Wei X, Chen J-H, Yang X, Li Z-B, et al. Elevated neutrophil to lymphocyte ratio predicts overall and cardiovascular mortality in maintenance peritoneal dialysis patients. *Int Urol Nephrol.* (2012) 44:1521–8. doi: 10.1007/s11255-012-0130-3
35. Cowan KJ, Storey KB. Mitogen-activated protein kinases: new signaling pathways functioning in cellular responses to environmental stress. *J Exp Biol.* (2003) 206:1107–15. doi: 10.1242/jeb.00220
36. de Heer E, Peters DJM. Innate immunity as a driving force in renal disease. *Kidney Int.* (2008) 73:7–8. doi: 10.1038/sj.ki.5002658
37. Rivera A, Siracusa MC, Yap GS, Gause WC. Innate cell communication kick-starts pathogen-specific immunity. *Nat Immunol.* (2016) 17:356–63. doi: 10.1038/ni.3375
38. IQWiG (Institute for Quality and Efficiency in Health Care). *The Innate and Adaptive Immune Systems*. InformedHealth.org (2020). Available online at: https://www.ncbi.nlm.nih.gov/books/NBK279396/
39. Novais FO, Scott P. Immunity to pathogens and tumors -proinflammatory cytokine - an overview. In: *Encyclopedia of Immunobiology 2016*. Science Direct Topics. Toronto, ON (2021).
40. Cano RLE, Lopera HDE. Introduction to T and B lymphocytes. In: *Autoimmunity: From Bench to Bedside*. Bogota: El Rosario University Press (2013).
41. Schnaper HW. The tubulointerstitial pathophysiology of progressive kidney disease. *Adv Chronic Kidney Dis.* (2017) 24:107–16. doi: 10.1053/j.ackd.2016.11.011
42. Matovinovic MS. Pathophysiology and classification of kidney disease. *EJIFCC*. (2009) 20:2–11.
43. De Haij S, Woltman AM, Trouw LA, Bakker AC, Kamerling SW, Van Der Kooij SW, et al. Renal tubular epithelial cells modulate T-cell responses via ICOS-L and B7-H1. *Kidney Int.* (2005) 68:2091–102. doi: 10.1111/j.1523-1755.2005.00665.x
44. Gong J, Noel S, Pluznick JL, Hamad ARA, Rabb H. Gut microbiota-kidney cross-talk in acute kidney injury. *Semin Nephrol*. (2019) 39:107–16. doi: 10.1016/j.semnephrol.2018.10.009
45. Alikhan MA, Huynh M, Kitching AR, Ooi JD. Regulatory T cells in renal disease. *Clin Transl Immunol.* (2018) 7:e1004. doi: 10.1002/cti2.1004
46. Mitsdoerffer M, Lee Y, Jäger A, Kim H-J, Korn T, Kolls JK, et al. Proinflammatory thelper type 17 cells are effective B-cell helpers. *Proc Natl Acad Sci USA*. (2010) 107:14292–7. doi: 10.1073/pnas.1009234107
47. Zheng L, Sinniah R, Hsu SI-H. Pathogenic role of NF-kappaB activation in tubulointerstitial inflammatory lesions in human lupus nephritis. *J Histochem Cytochem Off J Histochem Soc.* (2008) 56:517–29. doi: 10.1369/jhc.7A7368.2008
48. Hazenberg MD, Spits H. Human innate lymphoid cells. *Blood.* (2014) 124:700–9. doi: 10.1182/blood-2013-11-427781
49. Li S, Bostick JW, Zhou L. Regulation of innate lymphoid cells by aryl hydrocarbon receptor. *Front Immunol.* (2018) 8:1909. doi: 10.3389/fimmu.2017.01909
50. Krebs CF, Paust H-J, Krohn S, Koyro T, Brix SR, Riedel J-H, et al. Autoimmune renal disease is exacerbated by S1P-receptor-1-dependent intestinal Th17 cell migration to the kidney. *Immunity.* (2016) 45:1078–92. doi: 10.1016/j.immuni.2016.10.020
51. Luu M, Monning H, Visekruna A. Exploring the molecular mechanisms underlying the protective effects of microbial SCFAs on intestinal tolerance and food allergy. *Front Immunol.* (2020) 11:1225. doi: 10.3389/fimmu.2020.01225
52. Mielenz M, Kuhla B, Hammon HM. Abundance of adiponectin system and G-protein coupled receptor GPR109A mRNA in adipose tissue and liver of F2 offspring cows of Charolais × German Holstein crosses that differ in body fat accumulation. *J Dairy Sci.* (2013) 96:278–89. doi: 10.3168/jds.2012-5816
53. Imig JD, Ryan MJ. Immune and inflammatory role in renal disease. *Compr Physiol.* (2013) 3:957–76. doi: 10.1002/cphy.c120028
54. Stavropoulou E, Kantartzi K, Tsigalou C, Konstantinidis T, Romanidou G, Voidarou C, et al. Focus on the gut–kidney axis in health and disease. *Front Med.* (2021) 7:e620102. doi: 10.3389/fmed.2020.620102
55. Kato S, Chmielewski M, Honda H, Pecoits-Filho R, Matsuo S, Yuzawa Y, et al. Aspects of immune dysfunction in end-stage renal disease. *Clin J Am Soc Nephrol.* (2008) 3:1526–33. doi: 10.2215/CJN.00950208
56. Ando M, Shibuya A, Yasuda M, Azuma N, Tsuchiya K, Akiba T, et al. Impairment of innate cellular response to *in vitro* stimuli in patients on continuous ambulatory peritoneal dialysis. *Nephrol Dial Transplant.* (2005) 20:2497–503. doi: 10.1093/ndt/gfi048
57. Sester U, Sester M, Hauk M, Kaul H, Köhler H, Girndt M. T-cell activation follows Th1 rather than Th2 pattern in haemodialysis patients. *Nephrol Dial Transplant.* (2000) 15:1217–23. doi: 10.1093/ndt/15.8.1217
58. Eleftheriadis T, Antoniadi G, Liakopoulos V, Kartsios C, Stefanidis I. Basic science and dialysis: disturbances of acquired immunity in hemodialysis patients. *Semin Dial.* (2007) 20:440–51. doi: 10.1111/j.1525-139X.2007.00283.x
59. Sampani E, Stangou M, Daikidou DV, Nikolaidou V, Asouchidou D, Dimitriadis C, et al. P0297 changes of CD4CD28null and CD8CD28null T cells in chronic kidney disease and the impact of different dialysis modalities. *Nephrol Dial Transplant.* (2020) 35:gfaa142.P0297. doi: 10.1093/ndt/gfaa142.P0297
60. Nallu A, Sharma S, Ramezani A, Muralidharan J, Raj D. Gut microbiome in chronic kidney disease: challenges and opportunities. *Transl Res.* (2017) 179:24–37. doi: 10.1016/j.trsl.2016.04.007
61. Ramezani A, Raj DS. The gut microbiome, kidney disease, and targeted interventions. *J Am Soc Nephrol.* (2014) 25:657–70. doi: 10.1681/ASN.2013080905
62. Ardalan M, Vahed SZ. Gut microbiota and renal transplant outcome. *Biomed Pharmacother.* (2017) 90:229–36. doi: 10.1016/j.biopha.2017.02.114

63. Vossen JM, Guiot HFL, Lankester AC, Vossen ACTM, Bredius RGM, Wolterbeek R, et al. Complete suppression of the gut microbiome prevents acute graft-versus-host disease following allogeneic bone marrow transplantation. *PLoS ONE.* (2014) 9:e105706. doi: 10.1371/journal.pone.0105706
64. Lee JR, Muthukumar T, Dadhania D, Toussaint NC, Ling L, Pamer E, et al. Gut microbial community structure and complications after kidney transplantation: a pilot study. *Transplantation.* (2014) 98:697–705. doi: 10.1097/TP.0000000000000370
65. Lee JR, Muthukumar T, Dadhania D, Taur Y, Jenq RR, Toussaint NC, et al. Gut microbiota and tacrolimus dosing in kidney transplantation. *PLOS ONE.* (2015) 10:e0122399. doi: 10.1371/journal.pone.0122399
66. Coppo R. The gut–kidney axis in IgA nephropathy: role of microbiota and diet on genetic predisposition. *Pediatr Nephrol.* (2018) 33:53–61. doi: 10.1007/s00467-017-3652-1
67. Hu X, Du J, Xie Y, Huang Q, Xiao Y, Chen J, et al. Fecal microbiota characteristics of Chinese patients with primary IgA nephropathy: a cross-sectional study. *BMC Nephrol.* (2021) 21:97. doi: 10.1186/s12882-020-01741-9
68. Coppo R. The intestine-renal connection in IgA nephropathy. *Nephrol Dial Transplant.* (2015) 30:360–6. doi: 10.1016/j.semnephrol.2018.05.020
69. Zhang J, Luo D, Lin Z, Zhou W, Rao J, Li Y, et al. Dysbiosis of gut microbiota in adult idiopathic membranous nephropathy with nephrotic syndrome. *Microb Pathog.* (2020) 147:104359. doi: 10.1016/j.micpath.2020.104359
70. Gavazzi G, Krause K-H. Ageing and infection. *Lancet Infect Dis.* (2002) 2:659–66. doi: 10.1016/S1473-3099(02)00437-1
71. Fülöp T, Larbi A, Pawelec G. Human T Cell aging and the impact of persistent viral infections. *Front Immunol.* (2013) 4:271. doi: 10.3389/fimmu.2013.00271
72. Vasto S, Caruso C. Immunity & ageing: a new journal looking at ageing from an immunological point of view. *Immun Ageing.* (2004) 1:1. doi: 10.1186/1742-4933-1-1
73. Culley FJ. Natural killer cells in infection and inflammation of the lung. *Immunology.* (2009) 128:151–63. doi: 10.1111/j.1365-2567.2009.03167.x
74. Butcher S, Chahel H, Lord JM. Ageing and the neutrophil: no appetite for killing? *Immunology.* (2000) 100:411–6. doi: 10.1046/j.1365-2567.2000.00079.x
75. Yang J, Zhang L, Yu C, Yang X-F, Wang H. Monocyte and macrophage differentiation: circulation inflammatory monocyte as biomarker for inflammatory diseases. *Biomark Res.* (2014) 2:1. doi: 10.1186/2050-7771-2-1
76. Dunston CR, Griffiths HR. The effect of ageing on macrophage Toll-like receptor-mediated responses in the fight against pathogens. *Clin Exp Immunol.* (2010) 161:407–16. doi: 10.1111/j.1365-2249.2010.04213.x
77. Agrawal A, Sridharan A, Prakash S, Agrawal H. Dendritic cells and aging: consequences for autoimmunity. *Expert Rev Clin Immunol.* (2012) 8:73–80. doi: 10.1586/eci.11.77
78. Dunn AJ,Swiergiel AH. The role of cytokines in infection-related behavior. *Ann N Y Acad Sci.* (1998) 840:577–85. doi: 10.1111/j.1749-6632.1998.tb09596.x
79. Nikolich-Zugich J, Messaoudi I. Mice and flies and monkeys too: caloric restriction rejuvenates the aging immune system of non-human primates. *Exp Gerontol.* (2005) 40:884–93. doi: 10.1016/j.exger.2005.06.007
80. Lynch LA, O'Connell JM, Kwasnik AK, Cawood TJ, O'Farrelly C, O'Shea DB. Are natural killer cells protecting the metabolically healthy obese patient? *Obes Silver Spring Md.* (2009) 17:601–5. doi: 10.1038/oby.2008.565
81. Hersperger AR, Martin JN, Shin LY, Sheth PM, Kovacs CM, Cosma GL, et al. Increased HIV-specific CD8+ T-cell cytotoxic potential in HIV elite controllers is associated with T-bet expression. *Blood.* (2011) 117:3799–808. doi: 10.1182/blood-2010-12-322727
82. Garner ID. The impact of aging on susceptobility to infection. *Rev Inf Dis.* (1980) 2:801–10. doi: 10.1093/clinids/2.5.801
83. Lynch HE, Goldberg GL, Chidgey A, Van den Brink MRM, Boyd R, Sempowski GD. Thymic involution and immune reconstitution. *Trends Immunol.* (2009) 30:366–73. doi: 10.1016/j.it.2009.04.003
84. Walford RL. The immunologic theory of aging. *Immunol Rev.* (1969) 2:171–7. doi: 10.1111/j.1600-065X.1969.tb00210.x
85. Effros RB. Roy Walford and the immunologic theory of aging. *Immun Ageing A.* (2005) 2:7. doi: 10.1186/1742-4933-2-7
86. Freitas AA, de Magalhães JP. A review and appraisal of the DNA damage theory of ageing. *Mutat Res.* (2011) 728:12–22. doi: 10.1016/j.mrrev.2011.05.001
87. Aubert G, Lansdorp PM. Telomeres and aging. *Physiol Rev.* (2008) 88:557–79. doi: 10.1152/physrev.00026.2007
88. Shammas MA. Telomeres, lifestyle, cancer, and aging. *Curr Opin Clin Nutr Metab Care.* (2011) 14:28–34. doi: 10.1097/MCO.0b013e32834121b1
89. Capparelli C, Chiavarina B, Whitaker-Menezes D, Pestell TG, Pestell RG, Hulit J, et al. CDK inhibitors (p16/p19/p21) induce senescence and autophagy in cancer-associated fibroblasts, "fueling" tumor growth via paracrine interactions, without an increase in neo-angiogenesis. *Cell Cycle.* (2012) 11:3599–610. doi: 10.4161/cc.21884
90. Bello AK, Nwankwo E, El Nahas AM. Prevention on chronic kidney disease: a global challenge. *Kidney Int.* (2005) 68:S11–7. doi: 10.1111/j.1523-1755.2005.09802.x
91. Bikbov B, Purcell CA, Levey AS, Smith M, Abdoli A, Abebe M, et al. Global, regional, and national burden of chronic kidney disease, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet.* (2020) 395:709–33. doi: 10.1016/S0140-6736(20)30045-3
92. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis Off J Natl Kidney Found.* (2002) 39(2 Suppl. 1):S1–266.
93. Lin Lang C. A review of chronic kidney disease and the immune system: a special form of immunosenescence. *J Gerontol Geriat Res.* (2014) 3:2. doi: 10.4172/2167-7182.1000144
94. Liapis H, Romagnani P, Anders H-J. New Insights into the pathology of podocyte loss. *Am J Pathol.* (2013) 183:1364–74. doi: 10.1016/j.ajpath.2013.06.033
95. Weide T, Huber TB. Implications of autophagy for glomerular aging and disease. *Cell Tissue Res.* (2011) 343:467–73. doi: 10.1007/s00441-010-1115-0
96. Hartleben B, Gödel M, Meyer-Schwesinger C, Liu S, Ulrich T, Köbler S, et al. Autophagy influences glomerular disease susceptibility and maintains podocyte homeostasis in aging mice. *J Clin Invest.* (2010) 120:1084–96. doi: 10.1172/JCI39492
97. Efstratiadis G, Divani M, Katsioulis E, Vergoulas G. Renal fibrosis. *Hippokratia.* (2009) 13:224–9.
98. Linehan E, Fitzgerald DC. Ageing and the immune system: focus on macrophages. *Eur J Microbiol Immunol.* (2015) 5:14–24. doi: 10.1556/EuJMI-D-14-00035
99. Franceschi C, Garagnani P, Parini P, Giuliani C, Santoro A. Inflammaging: a new immune–metabolic viewpoint for age-related diseases. *Nat Rev Endocrinol.* (2018) 14:576–90. doi: 10.1038/s41574-018-0059-4

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

ORIGINAL RESEARCH
published: 26 March 2021
doi: 10.3389/fimmu.2021.621138

Clinical Significance of Shared T Cell Epitope Analysis in Early *De Novo* Donor-Specific Anti-HLA Antibody Production After Kidney Transplantation and Comparison With Shared B cell Epitope Analysis

Toshihide Tomosugi[1,2], Kenta Iwasaki[3], Shintaro Sakamoto[4], Matthias Niemann[5], Eric Spierings[6], Isao Nahara[7], Kenta Futamura[1], Manabu Okada[1], Takahisa Hiramitsu[1], Asami Takeda[8], Norihiko Goto[1], Shunji Narumi[1], Yoshihiko Watarai[1] and Takaaki Kobayashi[2]*

[1] Department of Transplant Surgery, Nagoya Daini Red Cross Hospital, Nagoya, Japan, [2] Department of Renal Transplant Surgery, Aichi Medical University School of Medicine, Nagakute, Japan, [3] Department of Kidney Diseases and Transplant Immunology, Aichi Medical University School of Medicine, Nagakute, Japan, [4] Department of Histocompatibility Laboratory, Nagoya Daini Red Cross Hospital, Nagoya, Japan, [5] PIRCHE AG, Berlin, Germany, [6] Center of Translational Immunology, UMC Utrecht, Utrecht, Netherlands, [7] Department of Pharmacoepidemiology, Kyoto University Graduate School of Medicine, School of Public Health, Kyoto, Japan, [8] Department of Nephrology, Nagoya Daini Red Cross Hospital, Nagoya, Japan

In pre-sensitizing events, immunological memory is mainly created *via* indirect allorecognition where $CD4^+$ T cells recognize foreign peptides in the context of self-HLA class II (pHLA) presented on antigen-presenting cells. This recognition makes it possible for naive $CD4^+$ T-helper cells to differentiate into memory cells, resulting in the creation of further antibody memory. These responses contribute to effective secretion of donor-specific anti-HLA antibodies (DSA) after second encounters with the same peptide. Preformed donor-reactive $CD4^+$ memory T cells may induce early immune responses after transplantation; however, the tools to evaluate them are limited. This study evaluated shared T cell epitopes (TEs) between the pre-sensitizing and donor HLA using an *in silico* assay, an alternative to estimate donor-reactive $CD4^+$ memory T cells before transplantation. In 578 living donor kidney transplants without preformed DSA, 69 patients had anti-HLA antibodies before transplantation. Of them, 40 had shared TEs and were estimated to have donor-reactive $CD4^+$ memory T cells. *De novo* DSA formation in the early phase was significantly higher in the shared TE-positive group than in the anti-HLA antibody- and shared TE-negative groups (p=0.001 and p=0.02, respectively). In conclusion, evaluation of shared TEs for estimating preformed donor-reactive $CD4^+$ memory T cells may help predict the risk of early *de novo* DSA formation after kidney transplantation.

Keywords: kidney transplantation, T cell epitope analysis, B cell epitope analysis, donor-specific antibody, PIRCHE-II

OPEN ACCESS

Edited by:
Frans H. J. Claas, Leiden University, Netherlands

Reviewed by:
Jean Villard, Université de Genève, Switzerland
Caner Süsal, Heidelberg University Hospital, Germany
Sandra Tafulo, Instituto Português de Sangue e da Transplantação, Portugal

****Correspondence:***
Toshihide Tomosugi
toshihidetomosugi67@gmail.com

Specialty section:
This article was submitted to Alloimmunity and Transplantation, a section of the journal Frontiers in Immunology

Received: *25 October 2020*
Accepted: *09 March 2021*
Published: *26 March 2021*

Citation:
Tomosugi T, Iwasaki K, Sakamoto S, Niemann M, Spierings E, Nahara I, Futamura K, Okada M, Hiramitsu T, Takeda A, Goto N, Narumi S, Watarai Y and Kobayashi T (2021) Clinical Significance of Shared T Cell Epitope Analysis in Early De Novo Donor-Specific Anti-HLA Antibody Production After Kidney Transplantation and Comparison With Shared B cell Epitope Analysis. Front. Immunol. 12:621138. doi: 10.3389/fimmu.2021.621138

INTRODUCTION

Adaptive immunity creates immunological memory after the first response to a specific foreign antigen; this memory leads to an enhanced rapid response to subsequent exposure to the same antigen and is important in organ transplantation (1). Immunological memory is created during pre-sensitizing events such as blood transfusion, pregnancy, and prior organ transplantation, and is reflected by the presence of anti-HLA antibodies (2). Production of antibody memory involves the indirect allorecognition pathway in which the T cell receptor of recipient naïve $CD4^+$ T-helper cells first recognizes a foreign-HLA-derived peptide in the context of recipient HLA class II (pHLA), which are presented on the recipient antigen-presenting cells (APCs) (3). Recipient naïve B cells, one of the APCs, also presents the foreign-HLA-derived pHLA, while specifically recognizing the foreign HLA with their B cell receptor. Unlike T cell receptors, B cell receptors recognize fragments on the tertiary structure of proteins, which are in structurally close contact (4). This indirect allorecognition with the foreign-HLA-specific T cell receptor allows naive $CD4^+$ T-helper cells to differentiate into memory cells, thereby helping naïve B cells with the foreign-HLA-specific B cell receptor to differentiate into antibody-producing plasma cells and memory B cells (5, 6). During this process, memory T cells and B cells memorize the molecular components known as epitope, not the foreign HLA as a whole (4). These memory cells lead an enhanced, rapid response after second encounters with the same epitopes derived from donor HLA (7–9).

The HLA loci have the most polymorphic regions in the human genome, with over 25,000 HLA alleles observed so far (10); however, the different HLA alleles often share epitopes with each other (11). Shared epitopes among multiple HLAs make it possible for memory cells to be recalled by not only the past-sensitizing HLAs, but also by the newly encountering HLAs, which creates a solid immunological defense system toward alloantigens (12). Reactivity toward shared epitopes was first confirmed in anti-HLA antibodies (13), whose recognizing region explained as B cell epitopes (BEs) were shared among multiple HLAs. The HLA groups shared with BEs were historically classified as cross-reactive-antigen groups (CREG) and received considerable attention as a risk predictor after transplantation. For example, organ allocation based on shared BEs, called CREG matching, was shown to reduce the frequency of sensitization to the donor HLA in a multicenter study (14). Furthermore, some recipients with preformed non-donor-specific anti-HLA antibodies (non-DSA) toward donor-CREG were reportedly associated with increased risks of early antibody-mediated rejection after transplantation (15). Recently, BEs in each HLA allele can be easily calculated using an *in silico* analytical tool known as the HLAMatchmaker. The analysis of shared BEs, such as CREG, has progressed to the current practices for solid organ transplantation. However, the pathological mechanisms by which organ allocation or risk stratification of non-DSA based on shared BEs affects the prognosis of transplant recipients remains unclear. These BE analyses could be a marker for structural similarity between each HLA molecule; however, these analyses are insufficient for estimating the mechanisms of acquired immunity because T cell reactivity is not considered in these methods. When focusing on the processes that create immunological memory, a joint approach of analyzing both BE and T cell epitope (TE) might give a comprehensive picture of pre-sensitization.

The reactivity of memory T cells toward shared TEs between the pre-sensitizing HLA and donor HLA may increase the risk of progression to early onset of rejection, resulting in poor graft prognosis (16); however, the tools to detect them are limited. Currently, enzyme-linked immunospot (ELISPOT) assay for the detection of allospecific cytokines produced by individual human peripheral blood lymphocytes is one of the main tools (17–19). Furthermore, detecting donor-HLA-reactive memory $CD4^+$ T cells *via* the indirect allorecognition pathway is technically difficult, although this pathway is thought to be a key mechanism in the progression of alloreactivity in organ transplantations (20).

Therefore, this study used the predicted indirectly recognizable HLA epitopes (PIRCHE)-II algorithm (21), an *in silico* assay focusing on the indirect allorecognition pathway, as an easy and alternative tool to estimate donor-reactive memory $CD4^+$ T cells. We hypothesized that the evaluation of shared TEs between the pre-sensitizing HLA and donor HLA for the purpose of estimating preformed donor-reactive memory $CD4^+$ T cells may be reasonable and helpful in predicting the risk of early *de novo* DSA (dnDSA) formation after transplantation (**Figure 1**), and we compared the efficacy of the risk predictor with the conventional evaluation of shared BEs.

MATERIALS AND METHODS

Patients

A total of 679 living donor kidney transplants from the Nagoya Daini Red Cross Hospital between 2012 and 2018 were eligible for this retrospective single-center cohort study. All recipients and donors were of Japanese origin. The final follow-up of all analyses was December 31, 2019. Informed consent was obtained from patients and donors in accordance with the Declaration of Helsinki. The study was approved by the Aichi Medical University Institutional Review Board.

Patient and Donor HLA Typing

Alleles at the HLA-A, -B, -DRB1, and -DQB1 loci were identified in all 679 pairs by xMAP® Technology of Luminex Corp. using PCR-sequence specific oligonucleotide (SSO) probes (WAKFlow HLA Typing kit, Wakunaga Pharmaceutical Co. Ltd., Hiroshima, Japan or One Lambda, Canoga Park, CA, USA) at high resolution. The typing kit can identify alleles with a frequency of 0.1% or more by combining the results with the information based on epidemiological allele frequency in the Japanese population (22). DRB3/4/5 and DQA1 were estimated using a local haplotype frequency dataset of 916 unrelated Japanese individuals (23). This estimation is widely used in the Japanese population because their haplotype frequencies have been concentrated based on a single ethnicity (24).

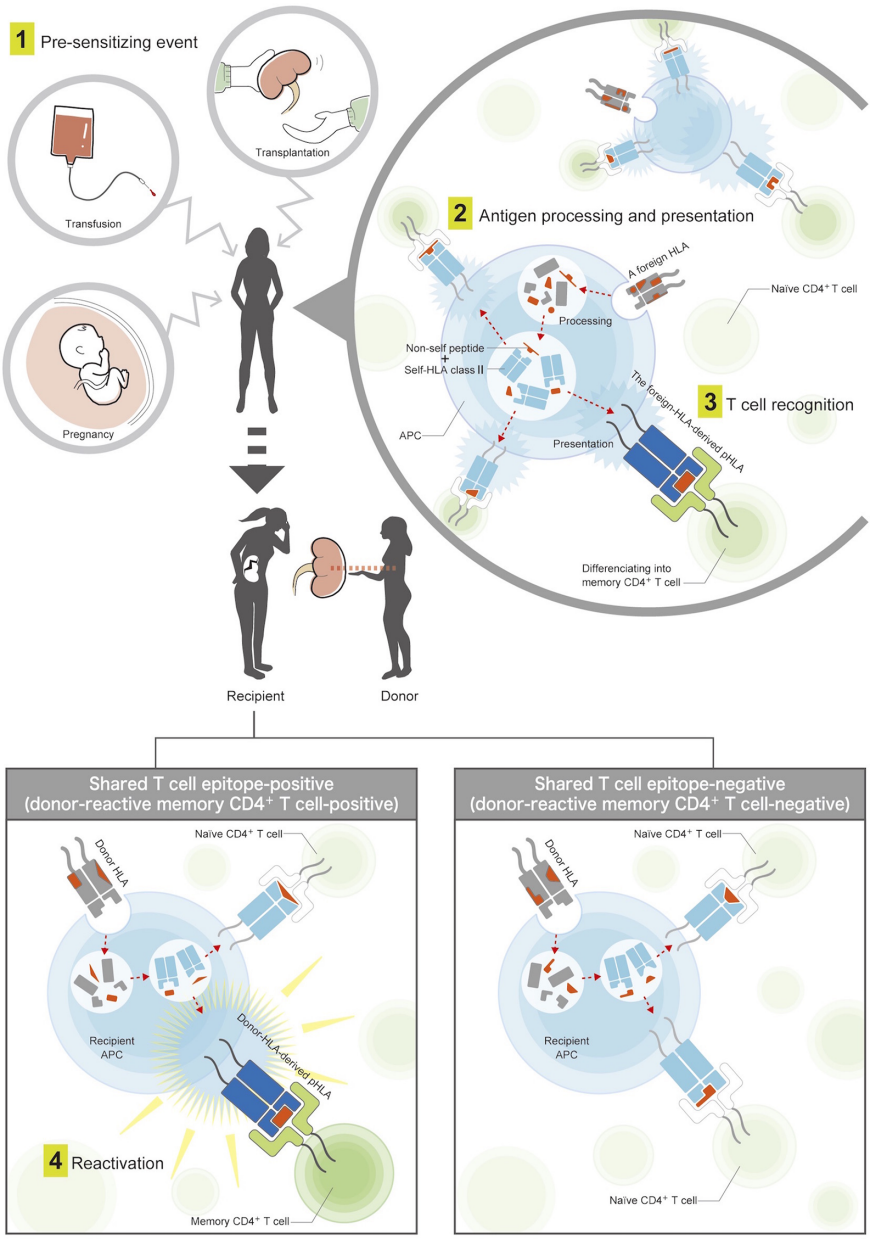


FIGURE 1 | Mechanisms of pre-sensitization *via* the indirect T cell-allorecognition pathway. (1) A patient is first exposed to foreign HLAs by pre-sensitizing events, such as blood transfusion, pregnancy, or prior organ transplantation. (2) The foreign HLAs are processed into smaller peptides by the patient's antigen-presenting cells. Among them, non-self peptides are loaded onto the recipient HLA class II, and the antigens are presented on the cell surface. (3) Patient's naïve $CD4^+$ T-helper cells recognize T cell epitopes consisting of the foreign-HLA-derived peptide in the context of recipient HLA class II (pHLA), which allows naive $CD4^+$ T-helper cells to differentiate into memory cells. (4) These memory $CD4^+$ T cells lead to an enhanced rapid response after second encounters with the same pHLAs derived from the donor HLA. The donor-reactive memory $CD4^+$ T-helper cells were considered to be positive if the foreign-HLA-derived pHLAs were shared with donor-HLA-derived pHLAs, and negative if not shared.

HLA Antibody Surveillance and Definition of Pre-Sensitizing HLA

Within the six months prior to transplantation, all patients were tested by complement-dependent cytotoxicity crossmatch (25), flow cytometry cross matches (26), and anti-HLA antibody screening (27, 28) with the use of flow panel reactive antibody (PRA) (One Lambda, California, US). Preformed DSA and non-DSA were determined by the Luminex-based LABScreen single antigen beads (SAB) assay (One Lambda, California, US). All serum samples used for antibody analysis were treated with EDTA to prevent the prozone effect. Mean fluorescence intensity (MFI) values >1000 were regarded as positive for the SAB assay.

In patients with preformed non-DSA, the HLA allele with the highest MFI value for each of HLA class I and class II before transplantation was considered as a pre-sensitizing HLA. All characteristics of non-DSA (MFI values >1000), as well as TE and BE counts shared with the donor HLA, are shown in **Supplementary Tables 1** and **2**. Post-transplantation anti-HLA antibody surveillance was annually performed by the flow PRA and SAB assays, according to the manufacturer's instructions. In cases of impaired allograft function, anti-HLA antibody surveillance was added accordingly. The HLA loci A, B, DRB1, DRB3/4/5, DQB1, and DQA1 were considered for the definition of DSA.

Shared TE-Analysis as a Tool to Estimate Donor-Reactive Memory CD4+ T-Helper Cells

The HLA-derived pHLAs, namely TEs, were all calculated using the latest version of the PIRCHE-II algorithm version 3.0 (PIRCHE AG, Berlin, Germany). HLA DRB1, DRB3/4/5, and DQB1/DQA1 were taken into consideration as presenting loci, while A, B, DRB1, DRB3/4/5, DQB1, and DQA1 were considered as presented loci. The binding probability of the presented peptide and presenting HLA class II was estimated; affinities with an IC50 of <1000 nM (27) were included in our analysis and the sum total of estimated TE-mismatch in each donor and recipient pair was defined as the PIRCHE-II score. The PIRCHE-II scores in this study were higher in range than those in previously reported (29, 30) in which only DRB1 was considered as the presenting locus. Based upon reports (29, 30), the natural logarithm of the PIRCHE-II scores [ln (PIRCHE-II)] were used to calculate the hazard ratio in the Cox proportional hazards regression model.

In patients with preformed non-DSA, TEs derived from pre-sensitizing HLA (see section *2.3* for details) were calculated using PIRCHE-II and compared with calculated TEs derived from the donor HLA. We considered the shared TEs to be positive and estimated the presence of donor-reactive memory $CD4^+$ T-helper cells if the two sets of TEs shared at least one pHLA, and negative if no pHLA were shared (**Figure 1**); for each non-DSA, TE counts shared with the donor HLA are shown in **Supplementary Table 1**.

HLAMatchmaker Analysis

BE mismatch levels for HLA-A, -B, -DRB1, -DRB3/4/5, and -DQB1/DQA1 were determined using the HLAMatchmaker software version 3.0 in each donor and recipient pair. The HLAMatchmaker score was considered as the total number of mismatched eplets, including antibody verified and non-verified eplets. The HLAMatchmaker score of 10 increments was also used to calculate the hazard ratio in the Cox proportional hazards regression model (29).

Shared BE Analysis

In patients with preformed non-DSA, similarly to the shared-TE analysis, the BEs derived from pre-sensitizing HLA (see section *2.3* for details) were estimated using the HLAMatchmaker software, and were compared with the calculated BEs derived from the donor HLAs. The shared BEs were determined as positive if the two sets of BEs shared at least one eplet, and negative if no eplets were shared; for each non-DSA, the BE counts shared with the donor HLA are shown in **Supplementary Table 2**.

Protocol Biopsies and Diagnosis of Rejection

Protocol biopsies were routinely performed on all patients at 2-3 weeks and 12 months after transplantation. In cases with impaired allograft function, biopsies were added accordingly. An experienced pathologist diagnosed antibody-mediated rejection and T cell-mediated rejection according to the revised Banff classification (31–34).

Immunosuppression

All patients received basiliximab as induction immunosuppression therapy. Patients who received an ABO-incompatible graft were additionally pretreated with rituximab and plasma exchange and/or double-filtration plasmapheresis before transplantation. Maintenance immunosuppression therapy consisted of triple therapy with prednisolone, calcineurin inhibitor (tacrolimus or cyclosporine), and mycophenolic acid. Some patients received the mammalian target of rapamycin inhibitor (everolimus) instead of mycophenolic acid.

Statistical Analysis

All statistical analyses were conducted using SPSS statistical software version 21 (IBM Corp., Armonk, NY). Continuous variables are expressed as a mean and standard deviation or median and interquartile range (IQR) according to their distribution and analyzed using the Student's *t*-test or Mann-Whitney *U* test. In cases of comparison across three groups, one-way analysis of variance (ANOVA) with the Kruskal-Wallis test was used. The Tukey honestly significant difference test was performed under the significant result of ANOVA for multiple comparisons. Categorical variables are expressed as a frequency and percentage and were examined using the Fisher exact or Chi-squared test according to the expected count. DSA-free graft survival was defined as the time between kidney transplantation and the date of the last anti-HLA antibody surveillance without DSA detection. Time-dependent outcomes such as DSA-free survival rates were estimated using the Kaplan-Meier survival curves and Breslow tests. The starting time point for these time-dependent survival analyses was determined as the day of transplantation. In the analysis of DSA-free survival, censoring occurred at the time of the last anti-HLA antibody surveillance. The Cox proportional hazards regression model for univariate analysis was used to find variables that affected DSA-free survival. Additionally, multivariate analysis with forced entry model was performed and adjusted for potential confounding factors that were selected based on the previous report (29) to assess the strength of the association after adjustment. P-values less than 0.05 were considered statistically significant. The relationships between the PIRCHE-II and HLAMatchmaker score and between the

shared-TE and shared-BE counts were investigated using the Spearman's rank-correlation coefficient (rho).

RESULTS

Patient Background

Consecutive living donor kidney transplants (n=679) were eligible for this study. We included only kidney transplants with complete HLA typing at high-resolution level (HLA-A, -B, -DRB1, and -DQB1) and pre- and post-transplantation follow-up for dnDSA surveillance. Twenty-six transplants were excluded because of incomplete post-transplantation DSA surveillance, and 27 patients whose HLA (HLA-A, -B, -DRB1, and -DQB1) were fully matched with the donors were excluded from the study (n=27) because dnDSA would not be detected in such patients; furthermore, 48 patients with preformed DSA were excluded. A total of 578 patients remained for analysis and were classified into either the preformed anti-HLA antibody-positive group (n=69) or anti-HLA antibody-negative group (n=509). The 69 HLA-sensitized transplants without preformed DSA were classified into either the shared TE-positive group (n=40) or shared TE-negative group (n=29) (**Figure 2**).

Table 1 describes the baseline characteristics of the shared TE-positive, shared TE-negative, and no anti-HLA antibody groups. The median follow-up period after transplantation was 47 months (IQR, 29-71.75 months; range, 1-95 months). Sensitizing events were seen more frequently in the HLA pre-sensitized (non-DSA) group, although there were no statistically significant differences (p=0.06). There were also no statistically significant differences in the other background characteristics including baseline immunosuppression therapy at transplantation, TE-mismatch count (calculated by PIRCHE-II), and BE-mismatch count (calculated by the HLAMatchmaker).

Characteristics of Estimated Pre-Sensitizing HLA

There were 69 HLA pre-sensitized (non-DSA) patients. Of these patients, 44 (63.8%) had only HLA class I, 15 (21.7%) had only HLA class II, and 10 (14.5%) had both HLA class I and II non-DSA. The median highest MFI of the preformed non-DSA before transplantation was 2,436.5 (IQR 1,462.75-6,134). Thirty-seven (53.6%) patients showed low-level MFI <3,000, 21 (30.4%) showed moderate-level MFI between 3,000 and 7,999, and 10 (14.5%) showed high-level MFI ≥8,000. Of these patient groups, there were no statistically significant differences in the characteristic of pre-sensitizing HLA between the shared TE-positive group (n=40) and shared TE-negative group (n=29), while the shared BE-positive status was seen more frequently in the shared TE-positive group (**Table 2**).

Characteristics of dnDSA

In this cohort, dnDSA were found in 52 of 578 patients (9.0%) during the full observational period, including HLA class I (n=5), DR (n=13), DQ (n=28), and DR+DQ (n=6). The median time to first detection was 26.5 months post-transplantation (IQR 11.75-37.5 months; range 0-84 months). Predominant dnDSA was directed against HLA class II (n=47), particularly DQ (n=34) and then DR (n=13). The incidence of class I DSA was low (n=5). The median highest MFI of dnDSA at the time of the first detection was 4,472.5 (IQR 2,070.5-1,1053.5) (**Table 3**).

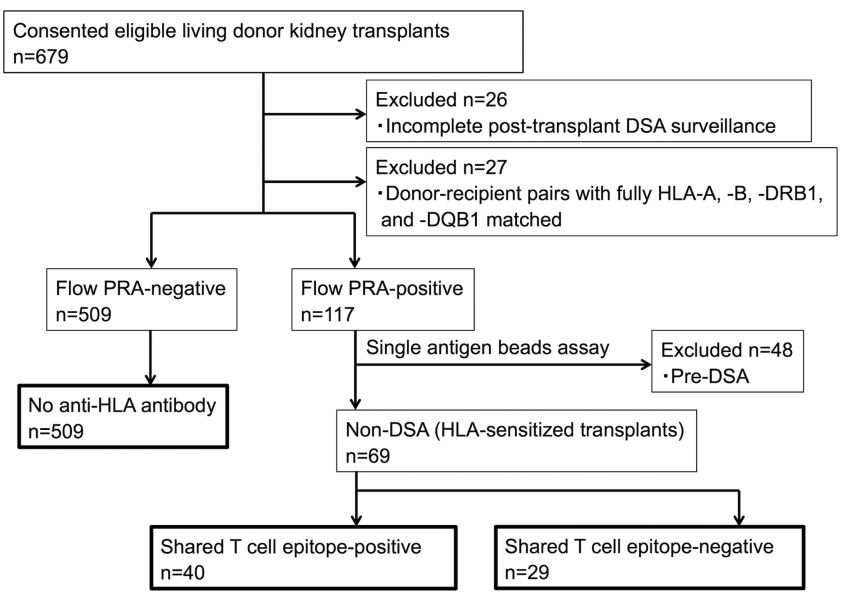


FIGURE 2 | Patient flowchart. Pre-DSA, preformed-donor-specific anti-HLA antibodies; non-DSA, non-donor-specific anti-HLA antibodies; PRA, panel reactive antibody.

TABLE 1 | Patient characteristics.

Characteristics	Anti-HLA ab (non-DSA)		No anti-HLA ab n = 509	*P*-value
	Shared TE-positive n = 40	Shared TE-negative n = 29		
Donor				
Age, years, mean (SD)	60.1 (9.3)	60.2 (10.0)	58.1 (10.1)	0.24
Female sex, n (%)	28 (70)	20 (69.0)	336 (66.0)	0.84
Relationship, n (%)				0.65
Unrelated	22 (55)	12 (41.4)	284 (55.8)	
Related (haplotype-unrelated)	2 (5)	2 (6.9)	21 (4.1)	
Related (haplotype-related*)	16 (40)	15 (51.7)	204 (40.1)	
Recipient				
Age, years, mean (SD)	49.5 (18.2)	49.0(12.8)	47.6 (16.3)	0.65
Female sex, n (%)	15 (37.5)	13 (44.8)	155 (30.5)	0.19
ABO-i, n (%)	14 (35)	9 (31.0)	184 (36.1)	0.85
ESRD causes, n (%)				
Glomerulonephritis	11 (27.5)	11 (37.9)	179 (35.2)	0.58
Polycystic kidney disease	4 (10)	1 (3.4)	33 (6.5)	0.54
Diabetes	10 (25)	5 (17.2)	116 (22.8)	0.74
Other	15 (37.5)	12 (41.4)	181 (35.6)	0.61
Months on dialysis, n (%)				0.47
0 (preemptive transplantation)	18 (45)	13 (44.8)	272 (53.4)	
-6	4 (10)	6 (20.7)	66 (13.0)	
6-47	10 (25)	8 (27.6)	108 (21.2)	
48-	8 (20)	2 (6.9)	63 (12.4)	
Pre-sensitizing event, n (%)**	19 (47.5)	14 (48.3)	170 (33.4)	0.06
Pre-transplantation	3 (7.5)	0 (0)	14 (2.8)	0.15
Pregnancy***	10 (66.7)	11 (84.6)	95 (61.3)	0.24
Transfusion	10 (25)	5 (17.2)	96 (18.9)	0.14
Histocompatibility (HLA-A, B, DRB1/3/4/5, DQB1, DQA1), median (IQR)				
HLA mismatches	6 (5 - 9)	5 (5 – 9)	6 (5 - 10)	0.34
HLAMatchmaker score				
AB	11 (5 - 16)	10 (4 – 16)	12 (7 - 16)	0.09
DR	8.5 (6 - 18)	7 (4 – 14)	11 (5 - 18)	0.51
DQ	13.5 (5 - 23)	12 (5 – 22)	13 (7 – 22)	0.92
Total	36 (24 – 49.5)	29 (19 – 41)	37 (25 – 51)	0.30
PIRCHE-II score	184.5 (120 – 280.5)	168 (112 - 260)	199 (131 – 298)	0.16
Baseline immunosuppression at transplant, n (%)				
Steroid	40 (100)	29 (100)	509 (100)	–
Tacrolimus	24 (60)	22 (75.9)	338 (66.4)	0.39
Cyclosporin	16 (40)	7 (24.1)	171 (33.6)	0.39
Everolimus	8 (20)	7 (24.1)	103 (20.2)	0.88
Micophenolic acid	31 (77.5)	22 (75.9)	400 (78.6)	0.93
Induction, n (%)				
Basiliximab	40 (100)	29 (100)	509 (100)	–
Thymoglobulin	0 (0)	0 (0)	0 (0)	–
Desensitization, n (%)				
Anti-CD20 therapy	11 (27.5)	7 (24.1)	154 (30.3)	0.74
Plasmapheresis	14 (35)	9 (31.0)	191 (37.5)	0.75
IVIG	0 (0)	0 (0)	2 (0.4)	0.87

**Haplotype-related donors shared one HLA haplotype with the recipients.*

***Pre-sensitizing events were recorded as 1 per patient, even if the patient had multiple pre-sensitizing events.*

****The percentage was calculated using only the female population.*

Ab, antibody; non-DSA, non-donor-specific anti-HLA antibodies; TE, T cell epitope; SD, standard deviation; ABO-I, ABO-incompatible transplantation; ESRD, end stage renal disease; PIRCHE, predicted indirectly recognizable HLA epitopes; IVIG, intravenous immunoglobulin; HLA, human leukocyte antigen; IQR, interquartile range.

Impact of the Shared TEs on dnDSA Formation: Comparison With the Shared BEs

To highlight the clinical impact of the pre-transplant memory $CD4^+$ T cells rather than the primary naïve immune response, we focused on the 3-year observational period after transplantation, which is a relatively early phase in the overall follow-up period of this study (median, 47 months; IQR, 29-71.75 months; range, 1-95 months). Within 3 years after organ transplantation, 38 patients were diagnosed with positive dnDSA. In this period, the non-DSA group tended to show higher incidences of dnDSA during the early phase after transplantation than the no anti-HLA antibody group, although this trend was not statistically significant (p=0.08) (**Figure 3A**). The non-DSA group was then divided into the shared

TABLE 2 | Characteristics of non-DSA with the highest MFI.

Characteristics of Non-DSA with the highest MFI value	Anti-HLA ab (non-DSA)		*P*-value
	Shared TE-positive n = 40	Shared TE-negative n = 29	
Non-DSA with the highest MFI value, n (%)			0.27
HLA class I	23 (57.5)	21 (72.4)	
HLA class II	9 (22.5)	6 (20.7)	
HLA class I & II	8 (20)	2 (6.9)	
The highest MFI value of non-DSA, n (%)			0.66
1000–2999	20 (50)	17 (58.6)	
3000–7999	13 (32.5)	9 (31.0)	
8000–	7 (17.5)	3 (10.3)	
Shared BE-positive, n (%)	34 (85)	13 (44.8)	<0.001

Ab, antibody; non-DSA, non-donor-specific anti-HLA antibodies; TE, T cell epitope; MFI, mean fluorescence intensity; BE, B cell epitope.

TABLE 3 | Characteristics of dnDSA in 578 patients without preformed DSA during the full observational period.

Characteristics of dnDSA	Anti-HLA ab (non-DSA)		No anti-HLA ab n = 509	*P*-value
	Shared TE-positive n = 40	Shared TE-negative n = 29		
dnDSA, n (%)	8 (20)	0 (0)	44 (8.6)	0.58
HLA class I	1 (2.5)	0 (0)	4 (0.8)	
HLA class II	7 (17.5)	0 (0)	40 (7.9)	
HLA class I & II	0 (0)	0 (0)	0 (0)	
The highest MFI value of dnDSA, n (%)				0.15
1000–2999	3 (7.5)	–	18 (3.5)	
3000–7999	4 (10)	–	10 (2.0)	
8000–	1 (2.5)	–	16 (3.1)	

Ab, antibody; TE, T cell epitope; dnDSA, de novo donor-specific anti-HLA antibodies; MFI, mean fluorescence intensity.

TE-positive and shared TE-negative groups. The shared TE-positive group showed significantly higher incidences of dnDSA than the shared TE-negative group (p=0.02) and no anti-HLA antibody group (p=0.001), while there was no statistically significant difference between the shared TE-negative group and no anti-HLA antibody group (p=0.19). The time to develop DSA after transplantation was statistically earlier in the shared TE-positive group than in the no anti-HLA antibody group (**Figure 3B**). We also checked the contributions of shared BEs on the development of dnDSA during the same period. Similar to the shared TE-positive group, the time to develop DSA after transplantation was statistically earlier in the shared BE-positive group than in the no anti-HLA antibody group; however, there were no statistically significant differences between the shared BE-positive and other groups in the analysis of DSA-free survival (**Figure 3C**).

Analysis on the Association Between the TE and BE

There was a moderately positive correlation between the TE-mismatch count (PIRCHE-II score) and the BE-mismatch count (HLAMatchmaker score), with a Spearman's rho of 0.68 ($p < 0.001$; **Figure 4A**). Conversely, the positive correlation between the shared-TE and shared-BE counts was weaker than that between the TE-mismatch and BE-mismatch counts (rho = 0.55, p<0.001) (**Figure 4B**). In each analysis, 3-year-dnDSA-positive patients (plotted in red) showed positive correlation to a lesser extent than all patients (TE- and BE-mismatch analysis; rho = 0.65, p<0.001 and shared-TE and -BE analysis; rho = 0.33, p = 0.006).

Risk Factors Associated With dnDSA Formation

During the 3-year observational period, shared TE-positive status, and ln(PIRCHE-II) were associated with an increased risk of dnDSA development in univariate Cox proportional hazards regression modeling. Furthermore, shared TE-positive status and ln(PIRCHE-II) remained significant in multivariate analysis. The PIRCHE-II score of shared TE-positive patients (median, 184.5; IQR, 120–280.5) was slightly lower than that of the total patients (median, 196; IQR, 129–291), and the adjustment for this difference might result in increased hazard ratio of shared TE-positive status in multivariate analysis. Shared BE-positive status was not associated with dnDSA formation in univariate Cox proportional hazards regression models (**Table 4-1**). We also included a longer observation period that spanned 5 years after transplantation. Univariate Cox proportional hazards regression modeling revealed that during this period, the shared TE-positive status, ln (PIRCHE-II), and HLAMatchmaker score were associated with an increased risk of dnDSA development. Although a longer observation period and HLA locus-specific analysis improve the power of the HLAMatchmaker score as a risk predictor for dnDSA formation, the shared TE-positive status and ln(PIRCHE-II) also remained significant risk factors in multivariate analysis (**Table 4-2**).

Cumulative Incidence of Adverse Outcomes in 5 Years

During the 5-year observational period after transplantation, cumulative dnDSA production occurred in 8 of 40 patients (20%) in the shared TE-positive group, in 0 of 29 patients (0%) in the shared TE-negative group, and in 42 of 509 (8.3%) patients in the no anti-HLA antibody group. The cumulative incidences of dnDSA were significantly higher in the shared TE-positive group compared with the other two groups during the full observational period; the onset appeared to be more frequent in the early phase, especially until 2 years after transplantation (p<0.001). Death-censored graft loss within 5 years after transplantation occurred in 3 (7.5%) patients in the shared TE-positive group, 0 (0%) patients in the shared TE-negative group, and 14 (2.8%) patients in the no anti-HLA antibody group (**Table 5**). There were no statistically significant differences in graft loss and rejection between these groups, although the number of the incidence of these events was low. Focusing on the 8 dnDSA cases in the shared TE-positive group, graft loss was observed in 2 patients, while antibody-mediated rejection was not observed within 5 years after transplantation; to evaluate the deleterious impact of dnDSA on the prognosis after transplantation, a longer observational period is required.

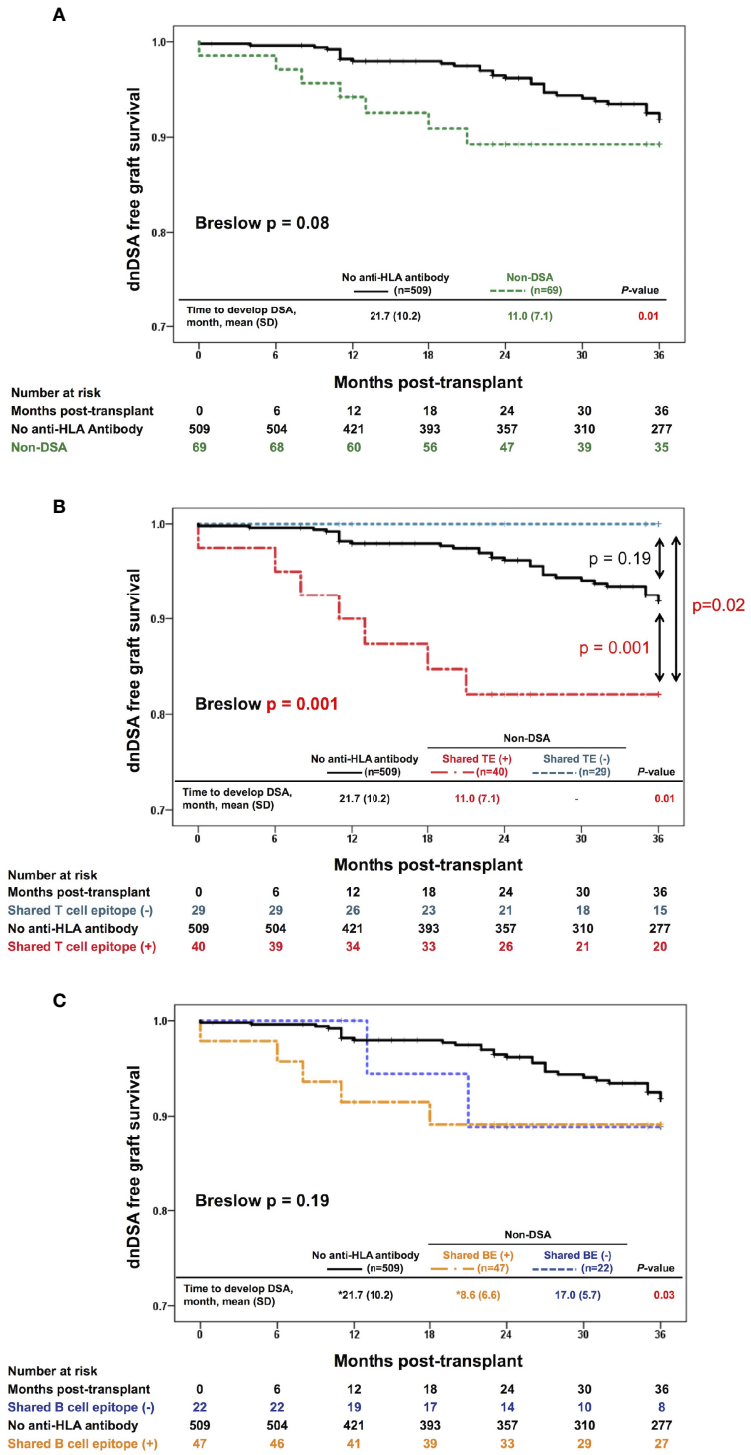


FIGURE 3 | Continued

FIGURE 3 | Effect of the shared TEs on dnDSA formation. **(A)** Three-year-dnDSA-free graft survival in the non-DSA versus no anti-HLA antibody group. The Kaplan-Meier curves and Breslow test tend to show higher incidences of dnDSA in the early phase after transplantation in the non-DSA group than in the no anti-HLA antibody group, although this trend is not statistically significant (p = 0.08). The time to develop DSA after transplantation is statistically earlier in the non-DSA group than in the no anti-HLA antibody group (p = 0.01). **(B)** Three-year-dnDSA-free graft survival in the three groups: shared TE-positive, shared TE-negative, and no anti-HLA antibody group. The Kaplan-Meier curves and Breslow test show a significant difference between these groups (p = 0.001). The shared TE-positive group shows significantly higher incidences of dnDSA than the no anti-HLA antibody group (p = 0.001), while there is no statistically significant difference between the shared TE-negative group and no anti-HLA antibody group (p = 0.19). The time to develop DSA after transplantation is statistically earlier in the shared TE-positive group than in the no anti-HLA antibody group (p = 0.01). **(C)** Three-year-dnDSA-free graft survival in three groups: shared BE-positive, shared BE-negative, and no anti-HLA antibody group. The Kaplan-Meier curves and Breslow test show no significant difference between these groups (p = 0.19). The time to develop DSA after transplantation is statistically different between these groups (p = 0.03). *Multiple comparison results show statistical differences between only the shared BE-positive and no anti-HLA antibody group. dnDSA, *de novo* donor-specific anti-HLA antibodies; non-DSA, non-donor-specific anti-HLA antibodies; TE, T cell epitope; BE, B cell epitope; SD, standard deviation.

Characteristics of Shared pHLAs in the Shared TE-Positive Group

Last, we focused on the shared TE-positive group. The details of shared pHLAs in 3-year-dnDSA-positive cases (n=7) are shown in **Table 6**. In three cases (patient number 29, 38, and 40), detected dnDSA were directed to the same HLA as the origin of the shared peptide; however, in the remaining 4 cases, the origin of the shared peptide was not the same HLA as the target of dnDSA. In comparison to the 3-year dnDSA-positive versus dnDSA-negative group in the shared TE-positive group, shared pHLAs tended to be derived from only HLA class I in the 3-year dnDSA-negative group, although this trend was not statistically significant (**Table 7**).

DISCUSSION

This study was the first attempt to use the PIRCHE-II algorithm as a tool to estimate preformed memory $CD4^+$ T cells, which may be reactivated by encountering pHLAs derived from the donor HLA *via* the indirect allorecognition pathway. As the first step in this study, pre-sensitizing HLA before transplantation had to be determined in order to assess shared TEs between the pre-sensitizing HLA and donor HLA. Clinically, it is difficult to determine this pre-sensitizing HLA by only considering a patient's medical history; thus, we focused on the characteristics of non-DSA as an objective tool to determine pre-sensitizing HLA. Anti-HLA antibodies toward a shared BE were reportedly diluted across multiple beads in a SAB assay, resulting in lowering the MFI values compared with antibodies toward a private epitope specific to a single HLA (35). Therefore, we considered that non-DSA with the highest MFI value may include antibodies toward private epitopes specific to pre-sensitizing HLA. As shown in **Supplementary Table 1**, the presence or absence of shared TEs between the donor HLA and non-DSA with the highest MFI value tended to represent the presence of shared TEs between the donor HLA and non-DSA with following ranks. In most cases in the shared TE-positive group (n=39/40), the non-DSA with the highest MFI, as well as the non-DSA with following ranks, shared TEs with the donor HLA. Furthermore, in the majority of the cases in the shared TE-negative group (n=23/29), both the non-DSA with the highest MFI and the non-DSA with following ranks did not share TEs with the donor HLA. These findings suggest that the use of non-DSA with the highest MFI as a predictor for pre-sensitizing HLA might be effective to some extent; however, we acknowledge that more cases are required for validation. Additionally, we analyzed the effect of shared BEs between the pre-sensitizing HLA and donor HLA on post-transplantation outcomes because the non-DSA toward BEs shared with the donor HLA were conventionally believed to be an immunological risk (15). In this study, 69 cases with non-DSA were analyzed and 47 cases were determined to share BEs between the pre-sensitizing HLA and donor HLA. As shown in **Supplementary Table 2**, the results for shared TEs did not always match the results for shared BEs. Some cases from the shared TE-positive group were determined to be shared BE-negative (n=6/40), while some cases from the shared TE-negative group were determined to be shared BE-positive (n=13/29). A previous report has suggested a moderately positive correlation between the TE-mismatch count (PIRCHE-II score) and the BE-mismatch count (HLAMatchmaker score) (29); in our study as well, a moderately positive correlation between the TE- and BE-mismatch counts was noted (**Figure 4A**). However, such a correlation was weakened between the shared-TE and shared-BE counts when the analysis was focused on the epitope shared between the non-DSA and donor HLA (**Figure 4B**). This weak correlation may cause 69 cases with non-DSA to be stratified differently between shared TE and BE, and lead to different outcomes on early dnDSA formation. In this cohort, the time to develop DSA after transplantation was statistically earlier in the shared BE-positive group than in the no anti-HLA antibody group; however we could not ascertain that shared BEs are a significant risk factor for early dnDSA formation (**Figure 3C**; **Tables 4-1** and **4-2**). Instead, shared TEs between the pre-sensitizing HLA and donor HLA were suggested to be a significant risk of dnDSA formation, especially in the early phase, implying the contribution of preformed donor-reactive memory $CD4^+$ T cells rather than the primary naïve immune response (**Figure 3B**). Additionally, in the multivariable Cox proportional hazards models, the shared TEs were suggested to be an independent risk factor affecting early dnDSA formation together with ln(PIRCHE-II), which was previously reported to be a risk factor (29, 30) (**Table 4**). Importantly, in our study, the non-DSA group tended to show higher incidences of dnDSA in the early phase after transplantation than the no anti-HLA antibody group, although this trend was not statistically significant (p=0.08) (**Figure 3A**); it remains controversial whether or not preformed non-DSA and their varieties could be an immunological risk for impaired graft

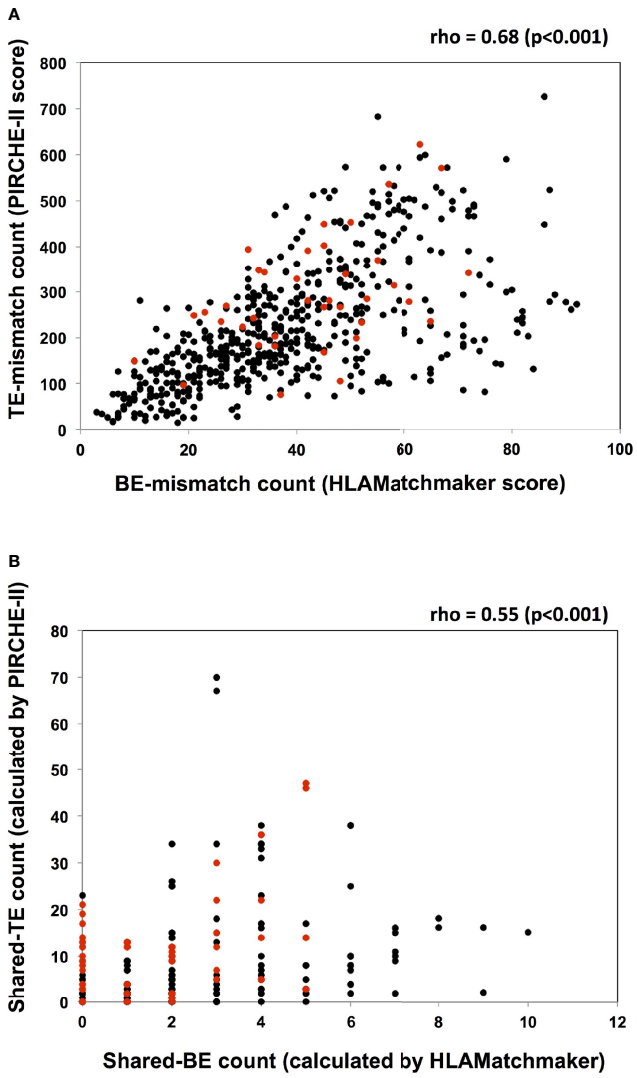


FIGURE 4 | Analyses of the association between TE and BE. **(A)** The TE-mismatch count (PIRCHE-II score) and the BE-mismatch count (HLAMatchmaker score) of each recipient and donor pair are plotted. A moderately positive correlation between the TE- and BE-mismatch counts is noted with a Spearman's rho of 0.68 ($p < 0.001$). Red circles and black circles indicate the dnDSA positive and negative patients, respectively. **(B)** The shared-TE count (calculated by PIRCHE-II) and the shared-BE count (calculated by HLAMatchmaker) between every detected non-DSA (MFI>1000) and the donor HLA are plotted. Each characteristic of non-DSA and the corresponding shared-TE and shared-BE counts are listed in **Supplementary Tables 1** and **2**. There is a weakly positive correlation between the shared-TE and shared-BE counts, with a Spearman's rho of 0.55 ($p < 0.001$). Red circles and black circles indicate non-DSA in dnDSA positive and negative patients, respectively. dnDSA, *de novo* donor-specific anti-HLA antibodies; non-DSA, non-donor-specific anti-HLA antibodies; TE, T cell epitope; BE, B cell epitope; PIRCHE, predicted indirectly recognizable HLA epitopes.

survival (36, 37). Our method clearly stratified non-DSA into deleterious or not deleterious by analyzing shared TEs. Additionally focusing on the shared TEs, PIRCHE-estimated-APC-presented peptides were not always derived from the same HLA as the target of dnDSA in the shared TE-positive group (**Table 6**). Similar to the shared TEs, HLAMatchmaker-estimated-shared BEs were not always derived from the same HLA as the target of dnDSA. For example, in one patient (shared-TE (+) - 5, in **Supplementary Table 2**), the shared TEs and BEs between the non-DSA and donor HLA were all derived from HLA class I, while the target of the detected dnDSA was HLA class II. This finding implied that the HLAs boosting memory $CD4^+$ T cells could be different from the HLAs that were targeted by antibodies. This could occur when patients were exposed to multiple HLAs during organ transplantation. These results supported our hypothesis that preformed donor-reactive memory $CD4^+$ T-helper cells activated by the shared TEs play a crucial role in promoting early dnDSA formation, even though there are no preformed DSA and their associated plasma cells and memory B cells.

However, we acknowledge that our methods may be a pseudomarker for T cell-responses, and this method has several limitations. First, this retrospective study in a single center features a relatively small sample size and brief observational period. Second, almost half of the patients with preformed non-DSA did not have any known pre-sensitizing event, which might be the result of an unrecognized pre-sensitizing event, such as early miscarriages in women or heterologous immunity (38); furthermore, it might be the result of false-positive SAB analysis (37, 39). Third, non-DSA with the highest MFI value may not always reflect actual pre-sensitizing history because most of the cases have been sensitized by multiple HLAs in repeated pre-sensitizing event. Furthermore, MFI values of SAB assays have analytic limitations in terms of quantitativeness of the antibody amount (35). There is still room for improving the *in silico* analysis to determine actual pre-sensitizing HLAs. Fourth, although it was reported that anti-HLA-C/-DP sensitization was also deleterious in kidney transplantation, HLA-C and DP were not typed and taken into account for the definition of DSA in this study (40). Fifth, this study lacks high-resolution HLA genotyping data on DRB3/4/5 and DQA1; these missing data were extrapolated to second field HLA typing using a local haplotype frequency dataset of 916 unrelated Japanese individuals (23). While we acknowledge that recent reports suggest insufficient accuracy of imputed HLA alleles, especially in ethnically heterogeneous non-Caucasian individuals (41), single ethnicity of our patients in this study would lower such the error rate. Sixth, we could only assess preformed donor-reactive memory in the non-DSA-positive population, since we did not have objective evidence except for anti-HLA antibodies. Detection of preformed donor-reactive memory in the no anti-HLA antibody group would be the next target. Considering these limitations, the validity of our findings needs to be confirmed by combining them with *in vitro* assays.

Although previous studies suggest that the standard *in vitro* assay of detecting preformed donor-reactive memory T cells was interferon gamma ELISPOT assay (IFNγ ELISPOT) (42), IFNγ ELISPOT can detect such T cells dominantly activated *via* the direct allorecognition pathway (43). In fact, pre-transplantation IFNγ ELISPOT positivity is broadly reported to be related to a high risk of rejection in the early phase (18, 42, 44). The *in silico* assay used in our study was especially focused on detecting preformed donor-reactive memory T cells activated *via* the indirect allorecognition pathway; our results showed that this method was related to a high risk of early dnDSA formation

TABLE 4-1 | Cox proportional hazards models of factors associated with dnDSA production in 3 years (n = 38).

Variables	Univariate analysis		*P*-value	Multivariate analysis		*P*-value
	HR	95% CI		HR	95% CI	
ABO-I vs ABO-Id/C	0.83	0.42–1.65	0.60			
Anti-CD20 use	0.91	0.44–1.87	0.79			
Pre-sensitizing event	0.80	0.41–1.59	0.53			
Count of HLA mismatches* per 1 increment	1.00	0.89–1.13	0.97			
Shared BE-positive	1.70	0.66–4.36	0.27			
Shared TE-positive**	3.37	1.48–7.65	0.004	3.80	1.66–8.67	0.002
ln(PIRCHE-II) score per 1 increment**	2.81	1.56–5.05	0.001	3.29	1.62–6.67	0.001
HLAMatchmaker score (A, B, DRB1/3/4/5, DQB1, and DQA1) per 10 increments	1.13	0.96–1.33	0.13			
HLAMatchmaker score (DRB1/3/4/5, DQB1, and DQA1) per 10 increments**	1.18	0.99–1.40	0.073	0.95	0.75–1.21	0.68

TABLE 4-2 | Cox proportional hazards models of factors associated with dnDSA production in 5 years (n = 50).

Variables	Univariate analysis		*P*-value	Multivariate analysis		*P*-value
	HR	95% CI		HR	95% CI	
ABO-I vs ABO-Id/C	0.63	0.34–1.19	0.15			
Anti-CD20 use	0.67	0.33–1.34	0.25			
Pre-sensitizing event	0.73	0.40–1.33	0.30			
Count of HLA mismatches* per 1 increment	1.02	0.92–1.13	0.66			
Shared BE-positive	1.54	0.66–3.61	0.32			
Shared TE-positive**	2.99	1.40–6.37	0.005	3.45	1.60–7.41	0.002
ln(PIRCHE-II) score per 1 increment**	2.71	1.64–4.50	<0.001	2.72	1.50–4.92	0.001
HLAMatchmaker score (A, B, DRB1/3/4/5, DQB1, and DQA1) per 10 increments	1.19	1.04–1.37	0.011			
HLAMatchmaker score (DRB1/3/4/5, DQB1, and DQA1) per 10 increments**	1.24	1.07–1.44	0.005	1.06	0.87–1.28	0.57

**HLA mismatch consists of mismatch at HLA-A, B, DRB1/3/4/5, DQB1, and DQA1 loci.*

***A multivariate analysis with forced entry model was generated using the univariate factors. Only HLAMatchmaker score (DRB1/3/4/5, DQB1, and DQA1) per 10 increments, ln(PIRCHE-II) score per 1 increment, and shared TE-positive were included in the multivariate analysis.*

dnDSA, de novo donor-specific anti-HLA antibody; HR, hazard ratio; CI, confidence interval; ABO-I, ABO-incompatible transplantation; ABO-Id/C, ABO-identical/compatible transplantation; non-DSA, non donor-specific anti-HLA antibody; BE, B cell epitope; TE, T cell epitope; PIRCHE, predicted indirectly recognizable HLA epitopes; ln (PIRCHE-II), natural logarithm of the PIRCHE-II scores.

TABLE 5 | Cumulative incidences of graft loss, rejection, and dnDSA.

Adverse outcomes	Anti-HLA ab (non-DSA)		No anti-HLA ab n = 509	*P*-value
	Shared TE-positive n = 40	Shared TE-negative n = 29		
Functional, n(%)				
5y graft loss	3 (7.5)	0 (0)	19 (3.7)	0.27
5y death censored graft loss	3 (7.5)	0 (0)	14 (2.8)	0.15
Immunological, n (%)				
5y antibody-mediated rejection	0 (0)	0 (0)	8 (1.6)	0.58
5y T cell-mediated rejection	2 (5)	1 (3.4)	29 (5.7)	0.87
5y dnDSA*	8 (20)	0 (0)	42 (8.3)	0.009
3y dnDSA*	7 (17.5)	0 (0)	31 (6.0)	0.007
2y dnDSA*	7 (17.5)	0 (0)	17 (3.3)	<0.001
1y dnDSA*	4 (10)	0 (0)	10 (2.0)	0.004

**Accumulating number of dnDSA-detected patients during each observational period, which include both persisting and disappearing dnDSA.*

Ab, antibody; non-DSA, non-donor-specific anti-HLA antibodies; TE, T cell epitope; 5/3/2/1y, 5/3/2/1-year; dnDSA, de novo donor-specific anti-HLA antibodies.

(**Figure 3**). In terms of the clinical effect on each T cell allorecognition pathway, these results are quite reasonable (45); however, validation *via in vitro* assays with a focus on the indirect allorecognition pathway is still required.

In addition to pre-transplantation risk stratification, further therapeutic consideration will be needed to reduce risk and improve prognosis, especially with the limited supply of organs. A previous report suggested that anti-thymocyte globulin (ATG) has the potential to control donor-reactive memory T cells detected by IFNγ ELISPOT (42). Although further clinical trial is required, ATG could be a beneficial intervention, even in patients with donor-reactive memory T cells, which would be activated *via* the indirect allorecognition pathway.

In conclusion, the evaluation of shared TEs using the PIRCHE-II algorithm for the purpose of estimating preformed donor-reactive memory $CD4^+$ T cells may help to predict the risk of early dnDSA formation after transplantation. Focusing on the pathogenesis of dnDSA formation, analysis of shared TEs is crucial for the precise understanding of the immune response to the donor organ, and should be distinguished from the conventional analysis of shared BEs. It remains difficult for *in vitro* assays to detect donor-reactive memory $CD4^+$ T cells activated *via* the indirect allorecognition pathway. Our study suggests that the *in silico*

TABLE 6 | Details of shared pHLAs; 3-year dnDSA-positive cases in the shared TE-positive group (n = 7).

Pt	DnDSA	Presenting HLA loci	Presented shared peptide	The origin of shared peptide	
No.				Pre-sensitizing HLA	Donor HLA
1	DQB1*06:04	DRB1*14:06	ITQRKWEAARVAEQL	B*54:01	A*33:03, B*44:03
		DRB1*14:06	QRKWEAARVAEQLRA	B*54:01	A*33:03, B*44:03
		DRB1*14:06	QLRAYLEGTCVEWLR	B*54:01	A*33:03
		DRB1*14:06	RAYLEGTCVEWLRRY	B*54:01	A*33:03
		DQA1*05:03 DQB1*03:01	QLRAYLEGTCVEWLR	B*54:01	A*33:03
		DQA1*05:03 DQB1*03:01	AQITQRKWEAARVAE	B*54:01	A*33:03, B*44:03
		DQA1*05:03 DQB1*03:01	QRKWEAARVAEQLRA	B*54:01	A*33:03, B*44:03
5	DRB3*03:01, DRB4*01:03	DQA1*01:03 DQB1*06:01	AQITQRKWEAARVAE	B*37:01	A*33:03, B*44:03
7	DQB1*03:01	DRB1*13:02	SMRYFYTSVSRPGRG	A*26:01	A*02:06
		DRB1*13:02	SHSMRYFYTSVSRPG	A*26:01	A*02:06
		DRB1*13:02	HSMRYFYTSVSRPGR	A*26:01	A*02:06
		DRB1*15:02	SMRYFYTSVSRPGRG	A*26:01	A*02:06
		DRB1*15:02	SHSMRYFYTSVSRPG	A*26:01	A*02:06
		DRB3*03:01	SHSMRYFYTSVSRPG	A*26:01	A*02:06
		DRB3*03:01	MRYFYTSVSRPGRGE	A*26:01	A*02:06
		DRB3*03:01	HSMRYFYTSVSRPGR	A*26:01	A*02:06
		DRB5*01:02	AVVAAVMWRRKSSDR	A*26:01	A*02:06
		DRB5*01:02	YTSVSRPGRGEPRFI	A*26:01	A*02:06
		DRB5*01:02	KETLQRTDAPKTHMT	A*26:01	A*02:06
		DRB5*01:02	SMRYFYTSVSRPGRG	A*26:01	A*02:06
		DRB5*01:02	MRYFYTSVSRPGRGE	A*26:01	A*02:06
		DQA1*01:02 DQB1*06:04	SHSMRYFYTSVSRPG	A*26:01	A*02:06
		DQA1*01:03 DQB1*06:01	SHSMRYFYTSVSRPG	A*26:01	A*02:06
		DQA1*01:03 DQB1*06:01	SMRYFYTSVSRPGRG	A*26:01	A*02:06
		DQA1*01:03 DQB1*06:04	SHSMRYFYTSVSRPG	A*26:01	A*02:06
8	DRB4*01:03	DRB1*01:01	DIVADHVASYGVNLY	DQA1*05:01	DQA1*03:02
		DRB1*01:01	EDIVADHVASYGVNL	DQA1*05:01	DQA1*03:02
		DRB1*01:01	FDPQFALTNIAVLKH	DQA1*05:01	DQA1*03:02
		DRB1*01:01	ASYGVNLYQSYGPSG	DQA1*05:01	DQA1*03:02
		DRB1*01:01	VVNITWLSNGHSVTE	DQA1*05:01	DQA1*03:02
		DRB1*01:01	VADHVASYGVNLYQS	DQA1*05:01	DQA1*03:02
		DRB1*15:02	QFALTNIAVLKHNLN	DQA1*05:01	DQA1*03:02
		DRB1*15:02	PVVNITWLSNGHSVT	DQA1*05:01	DQA1*03:02
		DRB1*15:02	FDPQFALTNIAVLKH	DQA1*05:01	DQA1*03:02
		DRB1*15:02	PQFALTNIAVLKHNL	DQA1*05:01	DQA1*03:02
		DRB1*15:02	ADHVASYGVNLYQSY	DQA1*05:01	DQA1*03:02
		DRB5*01:02	QFALTNIAVLKHNLN	DQA1*05:01	DQA1*03:02
		DRB5*01:02	VVNITWLSNGHSVTE	DQA1*05:01	DQA1*03:02
		DQA1*01:01 DQB1*06:01	ITWLSNGHSVTEGVS	DQA1*05:01	DQA1*03:02
		DQA1*01:01 DQB1*06:01	TWLSNGHSVTEGVSE	DQA1*05:01	DQA1*03:02
		DQA1*01:01 DQB1*06:01	RSNSTAATNEVPEVT	DQA1*05:01	DQA1*03:02
		DQA1*01:01 DQB1*06:01	ADHVASYGVNLYQSY	DQA1*05:01	DQA1*03:02
		DQA1*01:01 DQB1*06:01	PQFALTNIAVLKHNL	DQA1*05:01	DQA1*03:02
		DQA1*01:01 DQB1*06:01	KRSNSTAATNEVPEV	DQA1*05:01	DQA1*03:02
		DQA1*01:01 DQB1*06:01	FDPQFALTNIAVLKH	DQA1*05:01	DQA1*03:02
		DQA1*01:01 DQB1*06:01	QFALTNIAVLKHNLN	DQA1*05:01	DQA1*03:02
		DQA1*01:01 DQB1*06:01	DIVADHVASYGVNLY	DQA1*05:01	DQA1*03:02
		DQA1*01:03 DQB1*05:01	ADHVASYGVNLYQSY	DQA1*05:01	DQA1*03:02
		DQA1*01:03 DQB1*05:01	VASYGVNLYQSYGPS	DQA1*05:01	DQA1*03:02
		DQA1*01:03 DQB1*06:01	NITWLSNGHSVTEGV	DQA1*05:01	DQA1*03:02
		DQA1*01:03 DQB1*06:01	IVADHVASYGVNLYQ	DQA1*05:01	DQA1*03:02
		DQA1*01:03 DQB1*06:01	VNITWLSNGHSVTEG	DQA1*05:01	DQA1*03:02
		DQA1*01:03 DQB1*06:01	TWLSNGHSVTEGVSE	DQA1*05:01	DQA1*03:02
		DQA1*01:03 DQB1*06:01	ADHVASYGVNLYQSY	DQA1*05:01	DQA1*03:02
		DQA1*01:03 DQB1*06:01	FDPQFALTNIAVLKH	DQA1*05:01	DQA1*03:02
		DQA1*01:03 DQB1*06:01	PQFALTNIAVLKHNL	DQA1*05:01	DQA1*03:02
		DQA1*01:03 DQB1*06:01	ITWLSNGHSVTEGVS	DQA1*05:01	DQA1*03:02
		DQA1*01:03 DQB1*06:01	WLSNGHSVTEGVSET	DQA1*05:01	DQA1*03:02
		DQA1*01:03 DQB1*06:01	DIVADHVASYGVNLY	DQA1*05:01	DQA1*03:02
29	A*01:01	DQA1*03:02 DQB1*03:03	ALNEDLRSWTAADMA	A*34:01	A*01:01

(Continued)

TABLE 6 | Continued

Pt	DnDSA	Presenting HLA loci	Presented shared peptide	The origin of shared peptide	
No.				Pre-sensitizing HLA	Donor HLA
		DQA1*05:03 DQB1*03:03	IALNEDLRSWTAADM	A*34:01	A*01:01
		DRB1*09:01	DTYCRHNYGVVESFT	DRB1*15:01	DRB1*04:03
		DQA1*03:02 DQB1*03:01	YCRHNYGVVESFTVQ	DRB1*15:01	DRB1*04:03
		DQA1*05:03 DQB1*03:01	YCRHNYGVVESFTVQ	DRB1*15:01	DRB1*04:03
		DQA1*05:03 DQB1*03:03	YCRHNYGVVESFTVQ	DRB1*15:01	DRB1*04:03
38	DRB1*04:01	DRB1*15:01	EVTVYPAKTQPLQHH	DRB1*09:01	DRB1*04:01
		DRB5*01:01	EVTVYPAKTQPLQHH	DRB1*09:01	DRB1*04:01
		DQA1*01:02 DQB1*06:02	RNGQEEKAGVVSTGL	DRB1*09:01	DRB4*01:02
40	DRB5*01:01,	DRB1*08:03	RFDPQFALTNIAVLK	DQA1*05:05	DQA1*03:03
	DQA1*03:03	DRB1*08:03	HVASYGVNLYQSYGP	DQA1*05:05	DQA1*03:03
		DRB1*08:03	NITWLSNGHSVTEGV	DQA1*05:05	DQA1*03:03
		DRB1*08:03	DIVADHVASYGVNLY	DQA1*05:05	DQA1*03:03
		DRB1*08:03	IKRSNSTAATNEVPE	DQA1*05:05	DQA1*03:03
		DRB1*08:03	QFALTNIAVLKHNLN	DQA1*05:05	DQA1*03:03
		DRB1*08:03	PVVNITWLSNGHSVT	DQA1*05:05	DQA1*03:03
		DRB1*08:03	ADHVASYGVNLYQSY	DQA1*05:05	DQA1*03:03
		DRB1*13:02	VVNITWLSNGHSVTE	DQA1*05:05	DQA1*03:03
		DRB1*13:02	EDIVADHVASYGVNL	DQA1*05:05	DQA1*03:03
		DRB1*13:02	QFALTNIAVLKHNLN	DQA1*05:05	DQA1*03:03
		DRB3*03:01	QFALTNIAVLKHNLN	DQA1*05:05	DQA1*03:03
		DRB3*03:01	RFDPQFALTNIAVLK	DQA1*05:05	DQA1*03:03
		DRB3*03:01	PVVNITWLSNGHSVT	DQA1*05:05	DQA1*03:03
		DRB3*03:01	EDIVADHVASYGVNL	DQA1*05:05	DQA1*03:03
		DRB3*03:01	ADHVASYGVNLYQSY	DQA1*05:05	DQA1*03:03
		DRB3*03:01	VASYGVNLYQSYGPS	DQA1*05:05	DQA1*03:03
		DQA1*01:03 DQB1*06:01	NITWLSNGHSVTEGV	DQA1*05:05	DQA1*03:03
		DQA1*01:03 DQB1*06:01	IVADHVASYGVNLYQ	DQA1*05:05	DQA1*03:03
		DQA1*01:03 DQB1*06:01	VNITWLSNGHSVTEG	DQA1*05:05	DQA1*03:03
		DQA1*01:03 DQB1*06:01	TWLSNGHSVTEGVSE	DQA1*05:05	DQA1*03:03
		DQA1*01:03 DQB1*06:01	ADHVASYGVNLYQSY	DQA1*05:05	DQA1*03:03
		DQA1*01:03 DQB1*06:01	FDPQFALTNIAVLKH	DQA1*05:05	DQA1*03:03
		DQA1*01:03 DQB1*06:01	PQFALTNIAVLKHNL	DQA1*05:05	DQA1*03:03
		DQA1*01:03 DQB1*06:01	ITWLSNGHSVTEGVS	DQA1*05:05	DQA1*03:03
		DQA1*01:03 DQB1*06:01	WLSNGHSVTEGVSET	DQA1*05:05	DQA1*03:03
		DQA1*01:03 DQB1*06:01	DIVADHVASYGVNLY	DQA1*05:05	DQA1*03:03
		DQA1*01:03 DQB1*06:04	IVADHVASYGVNLYQ	DQA1*05:05	DQA1*03:03
		DQA1*01:03 DQB1*06:04	EDIVADHVASYGVNL	DQA1*05:05	DQA1*03:03
		DQA1*01:03 DQB1*06:04	ADHVASYGVNLYQSY	DQA1*05:05	DQA1*03:03
		DQA1*01:02 DQB1*06:01	PVVNITWLSNGHSVT	DQA1*05:05	DQA1*03:03
		DQA1*01:02 DQB1*06:01	NITWLSNGHSVTEGV	DQA1*05:05	DQA1*03:03
		DQA1*01:02 DQB1*06:01	IVADHVASYGVNLYQ	DQA1*05:05	DQA1*03:03
		DQA1*01:02 DQB1*06:01	RSNSTAATNEVPEVT	DQA1*05:05	DQA1*03:03
		DQA1*01:02 DQB1*06:01	DPQFALTNIAVLKHN	DQA1*05:05	DQA1*03:03
		DQA1*01:02 DQB1*06:01	ITWLSNGHSVTEGVS	DQA1*05:05	DQA1*03:03
		DQA1*01:02 DQB1*06:01	ADHVASYGVNLYQSY	DQA1*05:05	DQA1*03:03
		DQA1*01:02 DQB1*06:01	KRSNSTAATNEVPEV	DQA1*05:05	DQA1*03:03
		DQA1*01:02 DQB1*06:01	FDPQFALTNIAVLKH	DQA1*05:05	DQA1*03:03
		DQA1*01:02 DQB1*06:01	PQFALTNIAVLKHNL	DQA1*05:05	DQA1*03:03
		DQA1*01:02 DQB1*06:01	WLSNGHSVTEGVSET	DQA1*05:05	DQA1*03:03
		DQA1*01:02 DQB1*06:01	DIVADHVASYGVNLY	DQA1*05:05	DQA1*03:03

(Continued)

TABLE 6 | Continued

Pt No.	DnDSA	Presenting HLA loci	Presented shared peptide	The origin of shared peptide	
				Pre-sensitizing HLA	Donor HLA
		DQA1*01:02 DQB1*06:04	FDPQFALTNIAVLKH	DQA1*05:05	DQA1*03:03
		DQA1*01:02 DQB1*06:04	IVADHVASYGVNLYQ	DQA1*05:05	DQA1*03:03
		DQA1*01:02 DQB1*06:04	EDIVADHVASYGVNL	DQA1*05:05	DQA1*03:03
		DQA1*01:02 DQB1*06:04	ADHVASYGVNLYQSY	DQA1*05:05	DQA1*03:03

dnDSA, de novo donor-specific anti-HLA antibodies; Pt, patient; pHLA, peptide in the context of recipient HLA class II; No., number; A, alanine; R, arginine; N, asparagine; D, aspartic acid; C, cysteine; Q, glutamine; E, glutamic acid; G, glycine; H, histidine; I, Isoleucine; L, leucine; K, lysine; M, methionine; F, phenylalanine; P, proline; S, serine; T, threonine; W, Tryptophan; Y, tyrosine; V, valine.

TABLE 7 | Characteristics of shared pHLAs; comparison of 3-year dnDSA-positive versus negative group in the shared TE-positive group.

	3y dnDSA-positive	3y dnDSA-negative	*P*-value
	n = 7	n = 33	
Total shared pHLAs count, median (IQR)	7 (3–34)	4 (2–13)	0.17
The origin of the shared pHLAs, n (%)			
Pre-sensitizing HLA			0.39
HLA class I	3 (42.9)	23 (69.7)	
HLA class II	3 (42.9)	7 (21.2)	
HLA class I & II	1 (14.3)	3 (9.1)	
Donor HLA			0.44
HLA class I	3 (42.9)	22 (66.7)	
HLA class II	3 (42.9)	7 (21.2)	
HLA class I & II	1 (14.3)	4 (12.1)	

dnDSA, de novo donor-specific anti-HLA antibodies; 3y, 3-year; TE, T cell epitope; pHLA, peptide in the context of recipient HLA class II; IQR, interquartile range.

assay using the PIRCHE-II algorithm may be an effective and alternative solution for estimating this pathway. Considering the various limitations in this study, a larger sample size and further clinical and basic scientific approaches will be needed to validate this emerging *in silico* assay.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Ethics Committees of Aichi Medical University Hospital and the Institutional Review Board of Nagoya Daini Red Cross Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

TT, KI, and TK designed the research. TT wrote the manuscript. TT, SS, KF, MO, TH, AT, NG, SN, YW, and TK performed the research. TT, MN, ES, and TK participated in data analysis. IN reviewed statistics. MN, ES, and TK reviewed the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by JSPS KAKENHI Grant Numbers 20H03818 and 20K20608.

ACKNOWLEDGMENTS

The authors thank Harue Fukami and Kohei Nishida of the Histocompatibility Laboratory at Nagoya Red Cross Hospital for their excellent technical assistance.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.621138/full#supplementary-material

REFERENCES

1. Netea MG, Quintin J, van der Meer JW. Trained immunity: A memory for innate host defense. *Cell Host Microbe* (2011) 9(5):355–61. doi: 10.1016/j.chom.2011.04.006
2. Resse M, Paolillo R, Pellegrino Minucci B, Costa D, Fiorito C, Santangelo M, et al. Effect of single sensitization event on human leukocyte antigen alloimmunization in kidney transplant candidates: A single-center experience. *Exp Clin Transpl* (2018) 16(1):44–9. doi: 10.6002/ect.2016.0292
3. Game DS, Lechler RI. Pathways of allorecognition: Implications for transplantation tolerance. *Transpl Immunol* (2002) 10(2-3):101–8. doi: 10.1016/s0966-3274(02)00055-2
4. Sanchez-Trincado JL, Gomez-Perosanz M, Reche PA. Fundamentals and methods for T- and B-cell epitope prediction. *J Immunol Res* (2017) 2017:2680160. doi: 10.1155/2017/2680160

5. Tangye SG, Tarlinton DM. Memory B cells: Effectors of long-lived immune responses. *Eur J Immunol* (2009) 39(8):2065–75. doi: 10.1002/eji.200939531
6. Goodnow CC, Vinuesa CG, Randall KL, Mackay F, Brink R. Control systems and decision making for antibody production. *Nat Immunol* (2010) 11 (8):681–8. doi: 10.1038/ni.1900
7. Rogers PR, Dubey C, Swain SL. Qualitative changes accompany memory T cell generation: Faster, more effective responses at lower doses of antigen. *J Immunol* (2000) 164(5):2338–46. doi: 10.4049/jimmunol.164.5.2338
8. Okada T, Cyster JG. B cell migration and interactions in the early phase of antibody responses. *Curr Opin Immunol* (2006) 18(3):278–85. doi: 10.1016/j.coi.2006.02.005
9. Nicosia M, Fairchild RL, Valujskikh A. Memory T cells in transplantation: Old challenges define new directions. *Transplantation* (2020) 104(10):2024–34. doi: 10.1097/TP.0000000000003169
10. Robinson J, Barker DJ, Georgiou X, Cooper MA, Flicek P, Marsh SGE. IPD-IMGT/HLA database. *Nucleic Acids Res* (2020) 48(D1):D948–55. doi: 10.1093/nar/gkz950
11. Duquesnoy RJ. HLAMatchmaker: A molecularly based algorithm for histocompatibility determination. I. description of the algorithm. *Hum Immunol* (2002) 63(5):339–52. doi: 10.1016/s0198-8859(02)00382-8
12. Mongkolsuk T, Ingsathit A, Worawichawong S, Jirasiritham S, Kitpoka P, Thammanichanond D. Shared molecular eplet stimulates acute antibody-mediated rejection in a kidney transplant recipient with low-level donor-specific antibodies: A case report. *Transplant Proc* (2014) 46(2):644–7. doi: 10.1016/j.transproceed.2013.10.058
13. Joysey VC, Wolf E. HLA-A-B and -C antigens, their serology and cross-reaction. *Br Med Bull* (1978) 34(3):217–22. doi: 10.1093/oxfordjournals.bmb.a071501
14. Crowe DO. The effect of cross-reactive epitope group matching on allocation and sensitization. *Clin Transpl* (2003) 17(Suppl 9):13–6. doi: 10.1034/j.1399-0012.17.s9.2.x
15. Nainani N, Singh N, Shanahan T, Damodar A, Parimoo N, Ummadi S, et al. Cross reactive epitope group antibodies in sensitized kidneys transplant recipients was associated with early acute antibody mediated rejection. *Transpl Immunol* (2009) 20(3):113–7. doi: 10.1016/j.trim.2008.10.009
16. Gorbacheva V, Fan R, Fairchild RL, Baldwin W, Valujskikh A. Memory CD4 T cells induce antibody-mediated rejection of renal allografts. *J Am Soc Nephrol* (2016) 27(11):3299–307. doi: 10.1681/ASN.2015080848
17. Heeger PS, Greenspan NS, Kuhlenschmidt S, Dejelo C, Hricik DE, Schulak JA, et al. Pretransplant frequency of donor-specific, IFN-gamma-producing lymphocytes is a manifestation of immunologic memory and correlates with the risk of posttransplant rejection episodes. *J Immunol* (1999) 163(4):2267–75.
18. Gebauer BS, Hricik DE, Atallah A, Bryan K, Riley J, Tary-Lehmann M, et al. Evolution of the enzyme-linked immunosorbent spot assay for post-transplant alloreactivity as a potentially useful immune monitoring tool. *Am J Transpl* (2002) 2(9):857–66. doi: 10.1034/j.1600-6143.2002.20908.x
19. Nather BJ, Nickel P, Bold G, Presber F, Schonemann C, Pratschke J, et al. Modified ELISPOT technique–highly significant inverse correlation of post-tx donor-reactive IFNgamma-producing cell frequencies with 6 and 12 months graft function in kidney transplant recipients. *Transpl Immunol* (2006) 16(3-4):232–7. doi: 10.1016/j.trim.2006.09.026
20. Honjo K, Xu X, Bucy RP. $CD4^+$ T-cell receptor transgenic T cells alone can reject vascularized heart transplants through the indirect pathway of alloantigen recognition. *Transplantation* (2004) 77(3):452–5. doi: 10.1097/01.TP.0000112937.12491.42
21. Otten HG, Calis JJ, Kesmir C, van Zuilen AD, Spierings E. Predicted indirectly recognizable HLA epitopes presented by HLA-DR correlate with the *de novo* development of donor-specific HLA IgG antibodies after kidney transplantation. *Hum Immunol* (2013) 74(3):290–6. doi: 10.1016/j.humimm.2012.12.004
22. Hashimoto S, Nakajima F, Imanishi T, Kawai Y, Kato K, Kimura T, et al. Implications of HLA diversity among regions for bone marrow donor searches in Japan. *HLA* (2020) 96(1):24–42. doi: 10.1111/tan.13881
23. Hashimoto M, Kinoshita T, Yamasaki M, Tanaka H, Imanishi T, Ihara H, et al. Gene frequencies and haplotypic associations within the HLA region in 916 unrelated Japanese individuals. *Tissue Antigens* (1994) 44(3):166–73. doi: 10.1111/j.1399-0039.1994.tb02375.x
24. Ikeda N, Kojima H, Nishikawa M, Hayashi K, Futagami T, Tsujino T, et al. Determination of HLA-A, -C, -B, -DRB1 allele and haplotype frequency in Japanese population based on family study. *Tissue Antigens* (2015) 85(4):252–9. doi: 10.1111/tan.12536
25. Mittal KK, Mickey MR, Singal DP, Terasaki PI. Serotyping for homotransplantation. 18. refinement of microdroplet lymphocyte cytotoxicity test. *Transplantation* (1968) 6(8):913–27. doi: 10.1097/00007890-196811000-00006
26. Karpinski M, Rush D, Jeffery J, Exner M, Regele H, Dancea S, et al. Flow cytometric crossmatching in primary renal transplant recipients with a negative anti-human globulin enhanced cytotoxicity crossmatch. *J Am Soc Nephrol* (2001) 12(12):2807–14.
27. Gibney EM, Cagle LR, Freed B, Warnell SE, Chan L, Wiseman AC. Detection of donor-specific antibodies using HLA-coated microspheres: Another tool for kidney transplant risk stratification. *Nephrol Dial Transpl* (2006) 21 (9):2625–9. doi: 10.1093/ndt/gfl202
28. Caro-Oleas JL, Gonzalez-Escribano MF, Gentil-Govantes MA, Acevedo MJ, Gonzalez-Roncero FM, Blanco GB, et al. Clinical relevance of anti-HLA donor-specific antibodies detected by luminex assay in the development of rejection after renal transplantation. *Transplantation* (2012) 94(4):338–44. doi: 10.1097/TP.0b013e31825ace2c
29. Lachmann N, Niemann M, Reinke P, Budde K, Schmidt D, Halleck F, et al. Donor-recipient matching based on predicted indirectly recognizable HLA epitopes independently predicts the incidence of *de novo* donor-specific HLA antibodies following renal transplantation. *Am J Transpl* (2017) 17(12):3076–86. doi: 10.1111/ajt.14393
30. Geneugelijk K, Niemann M, Drylewicz J, van Zuilen AD, Joosten I, Allebes WA, et al. PIRCHE-II is related to graft failure after kidney transplantation. *Front Immunol* (2018) 9:321. doi: 10.3389/fimmu.2018.00321
31. Mengel M, Sis B, Haas M, Colvin RB, Halloran PF, Racusen LC, et al. Banff 2011 meeting report: New concepts in antibody-mediated rejection. *Am J Transpl* (2012) 12(3):563–70. doi: 10.1111/j.1600-6143.2011.03926.x
32. Haas M, Sis B, Racusen LC, Solez K, Glotz D, Colvin RB, et al. Banff 2013 meeting report: Inclusion of c4d-negative antibody-mediated rejection and antibody-associated arterial lesions. *Am J Transpl* (2014) 14(2):272–83. doi: 10.1111/ajt.12590
33. Loupy A, Haas M, Solez K, Racusen L, Glotz D, Seron D, et al. The banff 2015 kidney meeting report: Current challenges in rejection classification and prospects for adopting molecular pathology. *Am J Transpl* (2017) 17(1):28–41. doi: 10.1111/ajt.14107
34. Haas M, Loupy A, Lefaucheur C, Roufosse C, Glotz D, Seron D, et al. The banff 2017 kidney meeting report: Revised diagnostic criteria for chronic active T cell-mediated rejection, antibody-mediated rejection, and prospects for integrative endpoints for next-generation clinical trials. *Am J Transpl* (2018) 18(2):293–307. doi: 10.1111/ajt.14625
35. Konvalinka A, Tinckam K. Utility of HLA antibody testing in kidney transplantation. *J Am Soc Nephrol* (2015) 26(7):1489–502. doi: 10.1681/ASN.2014080837
36. Malheiro J, Tafulo S, Dias L, Martins S, Fonseca I, Beirao I, et al. Impact on mid-term kidney graft outcomes of pretransplant anti-HLA antibodies detected by solid-phase assays: Do donor-specific antibodies tell the whole story? *Hum Immunol* (2017) 78(9):526–33. doi: 10.1016/j.humimm.2017.07.011
37. Wehmeier C, Honger G, Cun H, Amico P, Hirt-Minkowski P, Georgalis A, et al. Donor specificity but not broadness of sensitization is associated with antibody-mediated rejection and graft loss in renal allograft recipients. *Am J Transpl* (2017) 17(8):2092–102. doi: 10.1111/ajt.14247
38. D'Orsogna L, van den Heuvel H, van Kooten C, Heidt S, Claas FHJ. Infectious pathogens may trigger specific allo-HLA reactivity via multiple mechanisms. *Immunogenetics* (2017) 69(8-9):631–41. doi: 10.1007/s00251-017-0989-3
39. Otten HG, Verhaar MC, Borst HP, van Eck M, van Ginkel WG, Hene RJ, et al. The significance of pretransplant donor-specific antibodies reactive with intact or denatured human leucocyte antigen in kidney transplantation. *Clin Exp Immunol* (2013) 173(3):536–43. doi: 10.1111/cei.12127
40. Bachelet T, Martinez C, Del Bello A, Couzi L, Kejji S, Guidicelli G, et al. Deleterious impact of donor-specific anti-HLA antibodies toward HLA-Cw and HLA-DP in kidney transplantation. *Transplantation* (2016) 100(1):159–66. doi: 10.1097/TP.0000000000000821
41. Engen RM, Jedraszko AM, Conciatori MA, Tambur AR. Substituting imputation of HLA antigens for high-resolution HLA typing: Evaluation of a multiethnic population and implications for clinical decision making in transplantation. *Am J Transpl* (2021) 21(1):344–552. doi: 10.1111/ajt.16070

42. Hricik DE, Augustine J, Nickerson P, Formica RN, Poggio ED, Rush D, et al. Interferon gamma ELISPOT testing as a risk-stratifying biomarker for kidney transplant injury: Results from the CTOT-01 multicenter study. *Am J Transpl* (2015) 15(12):3166–73. doi: 10.1111/ajt.13401
43. Bestard O, Nickel P, Cruzado JM, Schoenemann C, Boenisch O, Sefrin A, et al. Circulating alloreactive T cells correlate with graft function in longstanding renal transplant recipients. *J Am Soc Nephrol* (2008) 19(7):1419–29. doi: 10.1681/ASN.2007050539
44. Gandolfini I, Crespo E, Baweja M, Jarque M, Donadei C, Luque S, et al. Impact of preformed T-cell alloreactivity by means of donor-specific and panel of reactive T cells (PRT) ELISPOT in kidney transplantation. *PLoS One* (2018) 13(7):e0200696. doi: 10.1371/journal.pone.0200696
45. Siu JHY, Surendrakumar V, Richards JA, Pettigrew GJ. T cell allorecognition pathways in solid organ transplantation. *Front Immunol* (2018) 9:2548. doi: 10.3389/fimmu.2018.02548

Conflict of Interest: MN is an employee of PIRCHE AG. The UMC Utrecht has filed a patent application on the prediction of an alloimmune response against mismatched HLA. ES is listed as inventor on this patent.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

REVIEW
published: 14 April 2021
doi: 10.3389/fmed.2021.675573

Uremia-Associated Immunological Aging and Severity of COVID-19 Infection

*Michiel G. H. Betjes**

Division of Nephrology and Transplantation, Department of Internal Medicine, Erasmus Medical Centre, Rotterdam, Netherlands

One year after the start of the COVID-19 pandemic it has become clear that some groups of individuals are at particular high risk of a complicated course of infection resulting in high morbidity and mortality. Two specific risk factors are most prominent, old age and the presence of co-morbidity. Recent studies have shown that patients with compromised renal function, especially those treated with renal replacement therapy or having received a kidney transplant are at a much higher risk for severe COVID infection and increased mortality. This may be in part due to the increased prevalence of co-morbid conditions in these patients but specific alterations in their immune system, reflecting premature immunological aging, may be equally important. In this review the different aspects, in particular thymus function and memory T cell expansion, of uremia-associated immunological aging are reviewed with respect to COVID 19 infection. In essence, the decreased generation of naïve T cells may be instrumental in suboptimal anti-viral immune responses while the relatively uncontrolled expansion of effector T cells may facilitate the feared phase of the COVID-19 infection with excessive and live-threatening inflammation of the lung parenchyma.

Keywords: uremia, chronic kidney disease, thymus, adaptive immunity, lymphopenia, immunological aging, COVID-19

OPEN ACCESS

Edited by:
Maria J. Stangou, Aristotle University of Thessaloniki, Greece

Reviewed by:
Natalia Stepanova, SI "Institute of Nephrology National Academy of Medical Science of Ukraine", Ukraine
Erasmia Sampani, Hippokration General Hospital Thessaloniki Greece, Greece

***Correspondence:**
Michiel G. H. Betjes
m.g.h.betjes@erasmusmc.nl

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *03 March 2021*
Accepted: *22 March 2021*
Published: *14 April 2021*

Citation:
Betjes MGH (2021) Uremia-Associated Immunological Aging and Severity of COVID-19 Infection. Front. Med. 8:675573. doi: 10.3389/fmed.2021.675573

UREMIA-ASSOCIATED IMMUNOLOGICAL AGING

General Aspects

End-stage renal disease is associated with increased risks for infections, cancer and a poor vaccination response to vaccines like Hepatitis B surface antigen (HBsAg) (1). The accumulation of uremic toxins and increased oxidative stress leads to a pro-inflammatory state which is believed to underlie the impaired immune system. Uremia affects all aspects of both the innate and adaptive immune system [reviewed in (1)]. Cell numbers of innate immune cells like monocytes and granulocytes are normal to increased. However, these cells have a more activated profile with expansion of, for example, the subset of pro-inflammatory monocytes CD14posCD16pos while their functionality may be comprised (2, 3). Dendritic cells are professional antigen presenting cells and at the crossroad of the innate and adaptive immune response. In particular the subset of lymphoid dendritic cells is affected by aging and uremia as opposed to the myeloid dendritic cells (4–7). These lymphoid dendritic cells produce large amounts of type 1 interferon and are key for adequate antiviral responses (8). In addition, there are less dendritic cells present in the skin and circulation which may contribute to a less efficient adaptive immune response (5, 9).

Progressive lymphopenia with relatively more highly differentiated memory T cells is observed in association with more advanced stages of chronic kidney failure (10–12). Tracking the anti-HBsAg T cells after vaccination in patients with renal failure showed an insufficient CD4 T cell response which correlated closely with an impaired serological response (13).

The changes within the adaptive immune system and consequences for immune responses closely resembles the effects of aging (**Figure 1**) (14). A shift in favor of myeloid vs. lymphoid precursor hematological stem cells in the bone marrow may be important (15). This process is driven by epigenetics which in turn is under the influence of systemic inflammation and oxidative stress as observed in end-stage renal failure (16, 17). However, the adaptive immune system is more broadly affected by aging with thymus involution as a major cause of a decreasing output of naïve T cells, in combination with increasing numbers of memory T cells and changes in the regulatory T cell compartment. The first two observations are consistently found in the elderly and patients with end-stage renal failure. The expansion of memory T cells in elderly individuals is usually associated with a slight increases in markers of systemic inflammation and therefore frequently named inflamm-aging (18).

The T cell system is studied the most intense in aging research as peripheral blood is an easily accessible source of abundant T cells and many assays are available to study phenotype, differentiation status and function of T cells. In fact, such an integrative analysis of the T cell system of ESRD patients showed immunological aging by an average of 15–20 years, meaning that the composition of the population of circulating T cells of a 50-year old hemodialysis patient resembles that of a 70-year old healthy individual (11, 19). Of note, the inter-individual variation is substantial and for instance individuals with a genetic background of longevity show less signs of immunological aging (20).

Thymus Function and Aging

The thymus is important in producing naïve T cells which all have a specific T cell receptor (21). Naïve T cells leaving the thymus are called recent thymus emigrants (RTE) and were positively selected for the capacity to interact with the HLA molecules of the antigen-presenting cells but deleted if this interaction was to strong, thereby preventing potential dangerous autoreactivity. In addition, regulatory T cells (Tregs) are also generated which are called natural Tregs (22). Essentially, the thymus continuously generates the enormous diversity of T cell receptors which is needed to combat efficiently the wide variety of pathogens that may be encountered while controlling autoreactive T cells (23).

Aging is invariably associated with involution of the thymus leading to a steady decline in RTEs. The RTEs can be detected in the circulation by, for example, expression of CD31 on naïve T cells and there is on average an almost linear relationship between decreasing numbers of CD31 positive naïve T cells and age (24, 25). Homeostatic proliferation, particularly of CD4 naïve T cells, is able to maintain the volume of the naïve T cell compartment but naïve CD8 T cells become severely depleted in the elderly (26–29). This may lead to a contraction of the TCR repertoire which in turn can limit the diversity and thereby the efficacy of

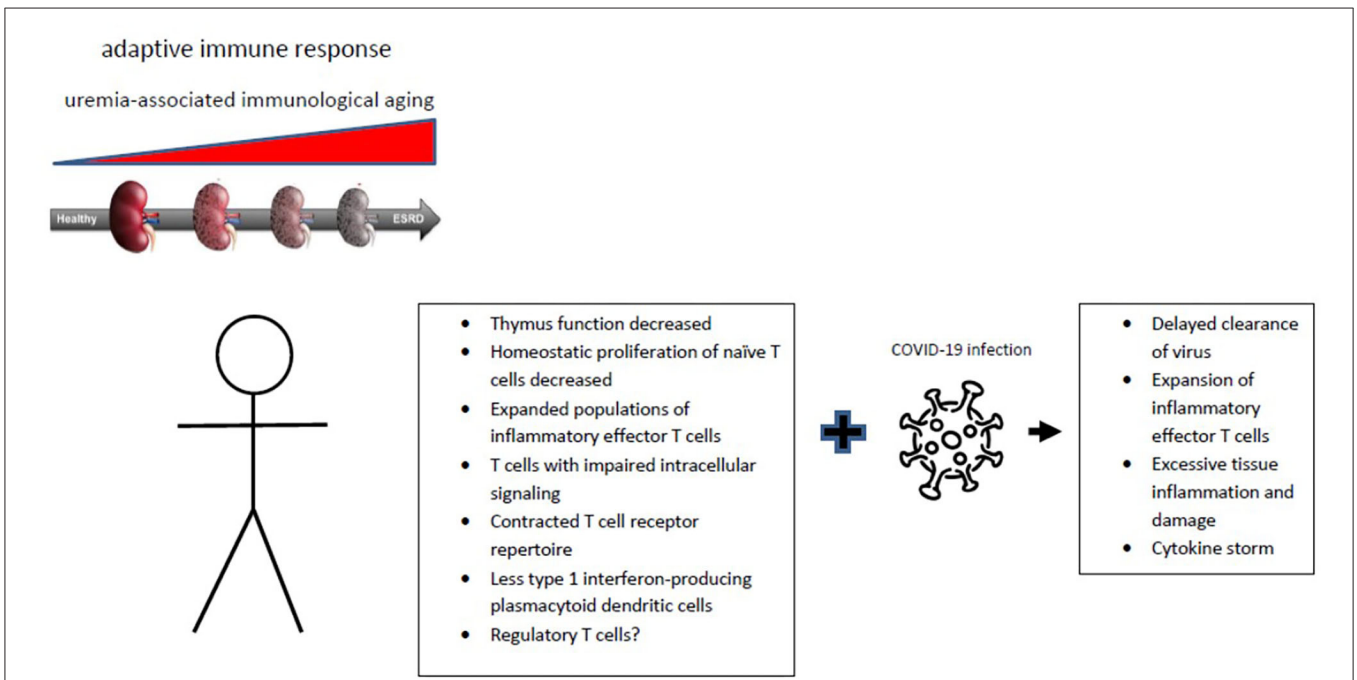


FIGURE 1 | Progression of chronic renal failure is associated with immunological aging affecting the adaptive immune system. The typical hall marks of immunological aging are given with an uncertain role for regulatory T cells. The changes of uremia-associated immunological aging can contribute to the substantially increased COVID-19 infection-associated morbidity and mortality of patients with chronic renal failure.

the immunological response (30). For instance, thymus output is crucial in the formation of protective immune responses during the early formation of a *Salmonella* infection but is dispensable once persistent *Salmonella* infection is established (31). Whether the output of natural Tregs is in line with the decrease in RTE's is not known.

Progressive severity of renal failure is associated with decreased numbers of naïve T cells which can be attributed to both a decrease in RTE's and a decrease in compensatory homeostatic proliferation (11, 32, 33). Activation-induced cell death of naïve T cells is increased and likely underlies part of the pathogenesis (11). As a result, at all decades of life end-stage renal failure results in a significant contraction of the circulating naïve T cell compartment (33, 34). Recent studies have shown that lymphoid and non-lymphoid tissues may also harbor naïve T cells and the relation with circulating naïve T cells is not clearly established (28, 35). However, comparing the percentage of lymph node naïve T cells with the percentage of peripheral blood naïve T cells showed a very close correlation (36).

Why the production of RTE's is affected by uremia is not known but from animal experiments it is clear that loss of renal function leads to volume loss of lymphoid organs like the thymus (37). The thymus and in particular the thymus epithelial cells appear to be very sensitive for inflammatory and oxidative stress which translates into an increased tendency for apoptosis (38–43). This may not only explain the thymus involution with normal aging, as a result of prolonged and variable exposure to these conditions, but also the uremia-associated decrease in thymus function in parallel with the increased pro-inflammatory environment observed with progressive loss of renal function.

Naïve T cell numbers in both healthy individuals as in recipients of kidney transplants were independently associated with all-cause mortality (19, 33, 44). Although this implies a causal relationship, this is not without uncertainty. Life events leading to episodes of increased inflammation and oxidative stress could accelerated thymus involution leading to "low for age" numbers of naïve T cells but also have a broad negative impact on the physical robustness of an individual. Therefore, the possibility exists that low naïve T cell numbers not only contribute to a weakened immune system but also point to a life history with harmful events leading to frailty (45–47).

Memory T Cell Expansion and Immunological Aging

The second hall mark of an aged T cell system is the expansion of memory T cells which may show signs of senescence and/or exhaustion which can be defined as the loss of proliferative capacity and specific effector functions like cytokine production and cytotoxicity (48). The increased numbers of memory T cells arise during life as a natural consequence of an immune system that has reacted to specific pathogens. When infections persist, like chronic hepatitis C or HIV, it may lead to progressive differentiation of virus-specific memory T cells into senescence and susceptibility for cell death (49–51). With increasing age, the memory T cells have undergone many rounds of replication with consequent shortening of their telomeres (52). Measuring telomere length therefore provides another measure of immunological aging (11).

Expansion of particular populations of memory T cells in the elderly persons may lead to a skewed TcR repertoire and may cause gaps in the TcR repertoire (53–56). The latter could fit in the concept of immunological space, which postulates that the immune system can only support the survival of a certain quantity of immune cells (57, 58). Of note, in recent years it has become evident that all tissues harbor a large quantity of resident T cells that do not circulate and that provide local protection against pathogens (59). As a first line of defense, the resident T cells are enriched in antigen-specific T cells that react to pathogens which are frequently encountered within that tissue, for example influenza-specific T cells in the lungs (60, 61). In contrast, relatively few highly differentiated T cells are present in the lymph nodes (36). Thus, the population of circulating T cells is only one of the many compartments of T cells, but easy to monitor and in general reflecting an ongoing immune response by increased frequencies of antigen-specific T cells.

Several studies have shown that an expanded population of differentiated effector memory T cells which have lost the expression of the co-stimulatory molecule CD28 is associated with less efficient vaccination and a decreased risk for rejection after kidney transplantation (14, 62–65). The underlying mechanisms may be multiple as the pool of CD28null T cells harbors many different cell types including senescent T cell and cells with a regulatory function (66, 67).

Circulating numbers of natural Tregs may increase with age as a result of an expanded population of memory Tregs. These inflated numbers of Tregs in the elderly can limit immune responses like vaccination response to influenza but could also foster autoimmunity and chronic inflammation (68). Chronic renal failure *per se* does not affect numbers and function of circulating natural Tregs (69).

Of interest is the observation from animal experiments and young adults after thymectomy at childhood, that lower numbers of naïve T cells facilitate an expansion of circulating memory T cells which may be a relevant phenomenon in immunological aging (70–72).

In patients with chronic renal failure the immunological aging of the memory T cells is more advanced as can be shown by the increased reduction in T cell telomere length and a higher frequency of highly differentiated T cells (2, 11, 19, 73). In addition, as in healthy individuals, the important intracellular signal pathway involving the MAP kinases ERK, p38 and DUSP6 is unfavorably changed by aging (74, 75).

IMPLICATIONS FOR COVID-19 INFECTION

Increasing morbidity and mortality associated with COVID-19 infection is highly associated with elderly age and co-morbid conditions (76). Patients on dialysis, with CKD and recipients of organ transplant represented three of the four comorbidities associated with the highest mortality risk from COVID-19 (77). Most likely, this is at least in part associated with their prematurely aged immune system as a coordinated adaptive T

cell response is associated with less severity of disease (78). Of note, changes at many levels of the immune system other than the adaptive T cell response have been described in association with aging and could contribute to severity of COVID-19 infection, but their relative importance has as yet not been established.

Fatality of COVID-19 infection is highly associated with a dysregulated immune response with progressive and severe inflammation of the lung parenchyma leading to extreme hypoxia (79–81). Several mutually not exclusive scenarios may lead to this outcome in the context of immunological aging. First, the aged immune system may be slow or inefficiently responding to this new viral pathogen as a result of a contracted TcR repertoire in the naïve T cell population and a general decline in T cell function by less effective intracellular signaling. In addition, the decreased numbers of plasmocytoid dendritic cells may have a profound negative effect on viral control as type 1 interferon is important in COVID-19 clearance in an experimental hamster model of infection (82).

Both deficiencies would lead to delayed clearance of the virus and prolonged stimulation and expansion of memory T cells that are COVID-19 reactive. On average lower numbers of T cells have been found in hospital-admitted COVID-19 patients and lower cell numbers, specifically naïve T cells, are related to disease severity (78, 83–88). Although of considerable interest, these observations are most likely caused by the COVID-19 infection itself which leads to decreased T cell numbers which may restore with clinical improvement (85, 89).

Second, immunological aging can lead to a T cell system prone to expansion of highly reactive memory T cells as regulation by Tregs is less efficient, low numbers of naïve T cells facilitate such a response and time to resolution of the viral infection is relatively slow. This scenario is not just hypothetical as shown by the large number of studies on infection with cytomegalovirus. Infection with this herpes virus typically leads to a strong T cell immune response which can be recognized by an expansion in the peripheral blood of both highly differentiated memory CD8 and CD4 T cells (90, 91). The CMV reactive CD4 T cells can be readily detected as they are negative for the co-stimulatory molecule CD28 (CD4posCD28null T cells)(90). Both the infectious dose and the age of the individual are related to the expansion of CMV-specific memory T cells (92, 93). Specifically, in elderly patients with chronic renal failure the expansion of CD4posCD28null T cells which normally comprise <1% of the CD4 T cell population may be such that over 50% of CD4 T cells become CD28null (94, 95). The CD4posCD28null T cells are highly cytotoxic and express the chemokine receptor CXCR3 which allows for migration over endothelial cells (96). These cells are not without harm as they have been identified as a non-classical risk factor for atherosclerotic disease probably by their capacity to destabilize atherosclerotic plaques (97, 98). Thus, CMV infection in immunologically aged individuals like patients with chronic renal failure may cause poorly controlled memory T cell expansion with subsequent collateral damage in patients with atherosclerotic plaques.

Such an exaggerated and harmful T cell immune response in elderly COVID-19 patients with a severe course of disease is of course much more acute and intense leading to expansion of highly activated memory T cells in association with a cytokine storm (88). In the case of COVID-19 the large expansion of highly reactive effector T cells is likely primarily present in the lung parenchyma as has, for example, been shown for influenza-specific T cells. Therefore, peripheral blood COVID-19 antigen-specific T cells are a reflection of the intensity of the immune response which may show different characteristics and may be much worse at the tissue level (99–102).

In the case of severe COVID-19 infection, controlling the inflammatory response by high dose steroid is currently the best option (103). A recent study among recipients of a liver transplant with COVID-19 infection showed that the use of tacrolimus was associated with a significant reduction of mortality (104). This findings underlines that limiting the excessive T cell response, in this case by tacrolimus, is a key element in harnessing the morbidity and mortality of COVID-19.

THERAPEUTIC STRATEGIES TO INFLUENCE UREMIA-ASSOCIATED IMMUNOLOGICAL AGING

Reversing immunological aging in humans is currently not possible although some interventions may be beneficial (40, 105). As thymus involution underlies ever decreasing naïve T cell numbers with aging and possible contributes to memory T cell expansion it would be of prime importance to control this process. The biological process of thymus involution is now better understood and it is clear that loss of thymus epithelial cells is essential.

Recent studies have shown that thymus involution involves the aging of the stromal microenvironment formed by thymus epithelial cells (TEC)(105). Many factors like cytokines, sex steroids and transcription factors are likely involved in TEC aging (106). Expression of the TEC autonomous transcription factor FOXN1 is pivotal for differentiation and maintaining TEC integrity. A null mutation of FOXN1 in mice results in a lack of hair and thymus, and gradual excision of FOXN1 over time in an experimental model results in thymus involution (107, 108).

This process can be favorably attenuated by transfecting thymus cells with FOXN1(70) and cellular therapy with FOXN1 producing stem cells or cytokine-to-TEC-based therapies using IL-22 or keratinocyte growth factor have shown promising results in experimental models. These approaches offer at least proof of the concept that thymus function can be (partially) restored (106).

Interleukin 7 is an important cytokine for T cell proliferation and homeostasis. Administration of recombinant IL-7 in humans appears to be safe and increases peripheral T cell numbers. However, there is little direct impact on thymus function which limits its use as a regenerative cytokine for the involuted thymus (109). Of interest, targeting of IL-7 to the thymus, for example, by a plasmid-delivered IL-7 fusion protein, was able to restore the thymus architecture and cellularity in aged animals (110).

Restoring renal function by kidney transplantation leads to a rapid clearance of inflammatory cytokines and relieves oxidative stress in ESRD patients. However, there is no reversal

in any of the markers of T cell aging even at 1 year after transplantation (111). Thus, once established, thymus involution seems irreversible, leaving the ESRD patient with premature aging at a persistent increased risk for mortality, even after regaining adequate renal function with a GFR over 60 mL/min. The underlying mechanisms are likely epigenetic changes induced by any combination of inflammation and oxidative stress associated with uremia, which are not easily reversible (1).

Of considerable interest is a recent observation that a healthy lifestyle may slow down thymus involution. Smoking and obesity are associated with fattening of the thymus (112) and bariatric surgery can partly reverse immunological aging (113). An observational study showed that elderly individuals with a high intensity of daily exercise had a better preservation of thymus function and less senescence of their immune system (114, 115). Having a healthy lifestyle with sufficient exercise will likely not reverse an atrophied thymus in ESRD patients but may delay further involution. Differences in lifestyle may also be part of the explanation for the substantial inter-individual variation observed at every decade of life in the number of naïve T cells and RTE's.

CONCLUSION

Aging of the T cell system has specific hall marks and is largely characterized by a progressive decrease of thymus function and expansion of highly differentiated memory T cells. Patients with renal failure, even after successful kidney transplantation may have severe premature immunological aging in particular in association with CMV infection. Immunological aging may explain why severity of COVID-19 infection is both age dependent and significantly increased in patients with chronic renal failure.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

REFERENCES

1. Betjes MG. Immune cell dysfunction and inflammation in end-stage renal disease. *Nat Rev Nephrol.* (2013) 9:255–65. doi: 10.1038/nrneph.2013.44
2. Chiu YL, Shu KH, Yang FJ, Chou TY, Chen PM, Lay FY, et al. A comprehensive characterization of aggravated aging-related changes in T lymphocytes and monocytes in end-stage renal disease: the iESRD study. *Immun Ageing.* (2018) 15:27. doi: 10.1186/s12979-018-0131-x
3. Girndt M, Trojanowicz B, Ulrich C. Monocytes in uremia. *Toxins.* (2020) 12:340. doi: 10.3390/toxins12050340
4. Jing Y, Shaheen E, Drake RR, Chen N, Gravenstein S, Deng Y. Aging is associated with a numerical and functional decline in plasmacytoid dendritic cells, whereas myeloid dendritic cells are relatively unaltered in human peripheral blood. *Hum Immunol.* (2009) 70:777–84. doi: 10.1016/j.humimm.2009.07.005
5. Hesselink DA, Betjes MG, Verkade MA, Athanassopoulos P, Baan CC, Weimar W. The effects of chronic kidney disease and renal replacement therapy on circulating dendritic cells. *Nephrol Dial Transplant.* (2005) 20:1868–73. doi: 10.1093/ndt/gfh897
6. Lim WH, Kireta S, Russ GR, Coates PT. Uremia impairs blood dendritic cell function in hemodialysis patients. *Kidney Int.* (2007) 71:1122–31. doi: 10.1038/sj.ki.5002196
7. Pérez-Cabezas B, Naranjo-Gómez M, Fernández MA, Grífols JR, Pujol-Borrell R, Borràs FE. Reduced numbers of plasmacytoid dendritic cells in aged blood donors. *Exp Gerontol.* (2007) 42:1033–8. doi: 10.1016/j.exger.2007.05.010
8. Reizis B. Plasmacytoid dendritic cells: development, regulation, and function. *Immunity.* (2019) 50:37–50. doi: 10.1016/j.immuni.2018.12.027
9. Mettang T, Fritz P, Weber J, Machleidt C, Hubel E, Kiefer T, et al. Epidermal Langerhans cells in uremic patients on hemodialysis or continuous ambulatory peritoneal dialysis. *Nephron.* (1993) 65:278–83. doi: 10.1159/000187488
10. Litjens NH, van Druningen CJ, Betjes MG. Progressive loss of renal function is associated with activation and depletion of naive T lymphocytes. *Clin Immunol.* (2006) 118:83–91. doi: 10.1016/j.clim.2005.09.007
11. Betjes MG, Langerak AW, van der Spek A, de Wit EA, Litjens NH. Premature aging of circulating T cells in patients with end-stage renal disease. *Kidney Int.* (2011) 80:208–17. doi: 10.1038/ki.2011.110
12. Yoon JW, Gollapudi S, Pahl MV, Vaziri ND. Naive and central memory T-cell lymphopenia in end-stage renal disease. *Kidney Int.* (2006) 70:371–6. doi: 10.1038/sj.ki.5001550
13. Litjens NH, Huisman M, van den Dorpel M, Betjes MG. Impaired immune responses and antigen-specific memory CD4+ T cells in hemodialysis patients. *J Am Soc Nephrol.* (2008) 19:1483–90. doi: 10.1681/ASN.2007090971
14. Crooke SN, Ovsyannikova IG, Poland GA, Kennedy RB. Immunosenescence and human vaccine immune responses. *Immun Ageing.* (2019) 16:25. doi: 10.1186/s12979-019-0164-9
15. Cho RH, Sieburg HB, Muller-Sieburg CE. A new mechanism for the aging of hematopoietic stem cells: aging changes the clonal composition of the stem cell compartment but not individual stem cells. *Blood.* (2008) 111:5553–61. doi: 10.1182/blood-2007-11-123547
16. Vaziri ND. Oxidative stress in uremia: nature, mechanisms, and potential consequences. *Semin Nephrol.* (2004) 24:469–73. doi: 10.1016/j.semnephrol.2004.06.026
17. Cohen G. Immune dysfunction in uremia 2020. *Toxins.* (2020) 12:439. doi: 10.3390/toxins12070439
18. Koelman L, Pivovarova-Ramich O, Pfeiffer AFH, Grune T, Aleksandrova K. Cytokines for evaluation of chronic inflammatory status in ageing research: reliability and phenotypic characterisation. *Immun Ageing.* (2019) 16:11. doi: 10.1186/s12979-019-0151-1
19. Crepin T, Legendre M, Carron C, Vachey C, Courivaud C, Rebibou JM, et al. Uraemia-induced immune senescence and clinical outcomes in chronic kidney disease patients. *Nephrol Dial Transplant.* (2020) 35:624–32. doi: 10.1093/ndt/gfy276
20. Derhovanessian E, Maier AB, Beck R, Jahn G, Hähnel K, Slagboom PE, et al. Hallmark features of immunosenescence are absent in familial longevity. *J Immunol.* (2010) 185:4618–24. doi: 10.4049/jimmunol.1001629
21. Thapa P, Farber DL. The role of the thymus in the immune response. *Thorac Surg Clin.* (2019) 29:123–31. doi: 10.1016/j.thorsurg.2018.12.001
22. Cebula A, Seweryn M, Rempala GA, Pabla SS, McIndoe RA, Denning TL, et al. Thymus-derived regulatory T cells contribute to tolerance to commensal microbiota. *Nature.* (2013) 497:258–62. doi: 10.1038/nature12079
23. Gallegos AM, Bevan MJ. Central tolerance: good but imperfect. *Immunol Rev.* (2006) 209:290–6. doi: 10.1111/j.0105-2896.2006.00348.x
24. Kohler S, Thiel A. Life after the thymus: CD31+ and CD31- human naive CD4+ T-cell subsets. *Blood.* (2009) 113:769–74. doi: 10.1182/blood-2008-02-139154
25. Ravkov E, Slev P, Heikal N. Thymic output: assessment of CD4(+) recent thymic emigrants and T-Cell receptor excision circles in infants. *Cytometry B Clin Cytom.* (2017) 92:249–57. doi: 10.1002/cyto.b.21341

26. van den Broek T, Borghans JAM, van Wijk F. The full spectrum of human naive T cells. *Nat Rev Immunol.* (2018) 18:363–73. doi: 10.1038/s41577-018-0001-y
27. Cicin-Sain L, Messaoudi I, Park B, Currier N, Planer S, Fischer M, et al. Dramatic increase in naive T cell turnover is linked to loss of naive T cells from old primates. *Proc Natl Acad Sci U S A.* (2007) 104:19960–5. doi: 10.1073/pnas.0705905104
28. Thome JJ, Grinshpun B, Kumar BV, Kubota M, Ohmura Y, Lerner H, et al. Longterm maintenance of human naive T cells through in situ homeostasis in lymphoid tissue sites. *Sci Immunol.* (2016) 1:eaah6506. doi: 10.1126/sciimmunol.aah6506
29. Fagnoni FF, Vescovini R, Passeri G, Bologna G, Pedrazzoni M, Lavagetto G, et al. Shortage of circulating naive CD8(+) T cells provides new insights on immunodeficiency in aging. *Blood.* (2000) 95:2860–8. doi: 10.1182/blood.V95.9.2860.009k35_2860_2868
30. Cicin-Sain L, Smyk-Pearson S, Currier N, Byrd L, Koudelka C, Robinson T, et al. Loss of naive T cells and repertoire constriction predict poor response to vaccination in old primates. *J Immunol.* (2010) 184:6739–45. doi: 10.4049/jimmunol.0904193
31. Goggins JA, Kurtz JR, McLachlan JB. Control of persistent salmonella infection relies on constant thymic output despite increased peripheral antigen-specific T cell immunity. *Pathogens.* (2020) 9:605. doi: 10.3390/pathogens9080605
32. Betjes MG, Litjens NH. Chronic kidney disease and premature ageing of the adaptive immune response. *Curr Urol Rep.* (2015) 16:471. doi: 10.1007/s11934-014-0471-9
33. Betjes MGH, Langerak AW, Klepper M, Litjens NHR. A very low thymus function identifies patients with substantial increased risk for long-term mortality after kidney transplantation. *Immun Ageing.* (2020) 17:4. doi: 10.1186/s12979-020-00175-z
34. Meijers RW, Litjens NH, de Wit EA, Langerak AW, van der Spek A, Baan CC, et al. Uremia causes premature ageing of the T cell compartment in end-stage renal disease patients. *Immun Ageing.* (2012) 9:19. doi: 10.1186/1742-4933-9-19
35. Thome JJ, Bickham KL, Ohmura Y, Kubota M, Matsuoka N, Gordon C, et al. Early-life compartmentalization of human T cell differentiation and regulatory function in mucosal and lymphoid tissues. *Nat Med.* (2016) 22:72–7. doi: 10.1038/nm.4008
36. Dedeoglu B, de Weerd AE, Huang L, Langerak AW, Dor FJ, Klepper M, et al. Lymph node and circulating T cell characteristics are strongly correlated in end-stage renal disease patients, but highly differentiated T cells reside within the circulation. *Clin Exp Immunol.* (2017) 188:299–310. doi: 10.1111/cei.12934
37. Raskova J, Czerwinski DK, Shea SM, Raska K, Jr. Cellular immunity and lymphocyte populations in developing uremia in the rat. *J Exp Pathol.* (1986) 2:229–45.
38. Cepeda S, Griffith AV. Thymic stromal cells: Roles in atrophy and age-associated dysfunction of the thymus. *Exp Gerontol.* (2018) 105:113–7. doi: 10.1016/j.exger.2017.12.022
39. Majumdar S, Adiga V, Raghavan A, Rananaware SR, Nandi D. Comparative analysis of thymic subpopulations during different modes of atrophy identifies the reactive oxygen species scavenger, N-acetyl cysteine, to increase the survival of thymocytes during infection-induced and lipopolysaccharide-induced thymic atrophy. *Immunology.* (2019) 157:21–36. doi: 10.1111/imm.13043
40. Majumdar S, Nandi D. Thymic atrophy: experimental studies and therapeutic interventions. *Scand J Immunol.* (2018) 87:4–14. doi: 10.1111/sji.12618
41. Paz-Miguel JE, Flores R, Sanchez-Velasco P, Ocejo-Vinyals G, Escribano de Diego J, Lopez de Rego J, et al. Reactive oxygen intermediates during programmed cell death induced in the thymus of the Ts(1716)65Dn mouse, a murine model for human Down's syndrome. *J Immunol.* (1999) 163:5399–410.
42. Venet F, Monneret G. Advances in the understanding and treatment of sepsis-induced immunosuppression. *Nat Rev Nephrol.* (2018) 14:121–37. doi: 10.1038/nrneph.2017.165
43. Venet F, Rimmele T, Monneret G. Management of Sepsis-Induced Immunosuppression. *Crit Care Clin.* (2018) 34:97–106. doi: 10.1016/j.ccc.2017.08.007
44. Courivaud C, Bamoulid J, Crepin T, Gaiffe E, Laheurte C, Saas P, et al. Pre-transplant thymic function predicts is associated with patient death after kidney transplantation. *Front Immunol.* (2020) 11:1653. doi: 10.3389/fimmu.2020.01653
45. Johnstone J, Parsons R, Botelho F, Millar J, McNeil S, Fulop T, et al. T-cell phenotypes predictive of frailty and mortality in elderly nursing home residents. *J Am Geriatr Soc.* (2017) 65:153–9. doi: 10.1111/jgs.14507
46. Reynolds CA, Tan Q, Munoz E, Jylhävä J, Hjelmborg J, Christiansen L, et al. A decade of epigenetic change in aging twins: genetic and environmental contributions to longitudinal DNA methylation. *Aging Cell.* (2020) 19:e13197. doi: 10.1111/acel.13197
47. Tan Q, Heijmans BT, Hjelmborg JV, Soerensen M, Christensen K, Christiansen L. Epigenetic drift in the aging genome: a ten-year follow-up in an elderly twin cohort. *Int J Epidemiol.* (2016) 45:1146–58. doi: 10.1093/ije/dyw132
48. Saule P, Trauet J, Dutriez V, Lekeux V, Dessaint JP, Labalette M. Accumulation of memory T cells from childhood to old age: central and effector memory cells in CD4(+) versus effector memory and terminally differentiated memory cells in CD8(+) compartment. *Mech Ageing Dev.* (2006) 127:274–81. doi: 10.1016/j.mad.2005.11.001
49. Brenchley JM, Karandikar NJ, Betts MR, Ambrozak DR, Hill BJ, Crotty LE, et al. Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of CD8+ T cells. *Blood.* (2003) 101:2711–20. doi: 10.1182/blood-2002-07-2103
50. Hove-Skovsgaard M, Zhao Y, Tingstedt JL, Hartling HJ, Thudium RF, Benfield T, et al. Impact of age and HIV status on immune activation, senescence and apoptosis. *Front Immunol.* (2020) 11:583569. doi: 10.3389/fimmu.2020.583569
51. Gindin Y, Gaggar A, Lok AS, Janssen HLA, Ferrari C, Subramanian GM, et al. DNA methylation and immune cell markers demonstrate evidence of accelerated aging in patients with chronic HBV or HCV, with or without HIV co-infection. *Clin Infect Dis.* (2020). doi: 10.1093/cid/ciaa1371. [Epub ahead of print].
52. Hadrup SR, Strindhall J, Kollgaard T, Seremet T, Johansson B, Pawelec G, et al. Longitudinal studies of clonally expanded CD8 T cells reveal a repertoire shrinkage predicting mortality and an increased number of dysfunctional cytomegalovirus-specific T cells in the very elderly. *J Immunol.* (2006) 176:2645–53. doi: 10.4049/jimmunol.176.4.2645
53. Ahmed M, Lanzer KG, Yager EJ, Adams PS, Johnson LL, Blackman MA. Clonal expansions and loss of receptor diversity in the naive CD8 T cell repertoire of aged mice. *J Immunol.* (2009) 182:784–92. doi: 10.4049/jimmunol.182.2.784
54. Naylor K, Li G, Vallejo AN, Lee WW, Koetz K, Bryl E, et al. The influence of age on T cell generation and TCR diversity. *J Immunol.* (2005) 174:7446–52. doi: 10.4049/jimmunol.174.11.7446
55. Huang L, Betjes MGH, Klepper M, Langerak AW, Baan CC, Litjens NHR. end-stage renal disease causes skewing in the TCR vbeta-repertoire primarily within CD8(+) T cell subsets. *Front Immunol.* (2017) 8:1826. doi: 10.3389/fimmu.2017.01826
56. Huang L, Langerak AW, Wolvers-Tettero IL, Meijers RW, Baan CC, Litjens NH, et al. End stage renal disease patients have a skewed T cell receptor Vbeta repertoire. *Immun Ageing.* (2015) 12:28. doi: 10.1186/s12979-015-0055-7
57. Callard RE, Stark J, Yates AJ. Fratricide: a mechanism for T memory-cell homeostasis. *Trends Immunol.* (2003) 24:370–5. doi: 10.1016/S1471-4906(03)00164-9
58. Naismith E, Pangrazzi L, Grasse M, Keller M, Miggitsch C, Weinberger B, et al. Peripheral antibody concentrations are associated with highly differentiated T cells and inflammatory processes in the human bone marrow. *Immun Ageing.* (2019) 16:21. doi: 10.1186/s12979-019-0161-z
59. Nanda NK. Tissue-resident memory T cells: sheltering-in-place for host defense. *Crit Rev Immunol.* (2020) 40:423–40. doi: 10.1615/CritRevImmunol.2020035522
60. Schmidt A, Lapuente D. T cell immunity against influenza: the long way from animal models towards a real-life universal flu vaccine. *Viruses.* (2021) 13:199. doi: 10.3390/v13020199

61. Laidlaw BJ, Zhang N, Marshall HD, Staron MM, Guan T, Hu Y, et al. CD4+ T cell help guides formation of CD103+ lung-resident memory CD8+ T cells during influenza viral infection. *Immunity.* (2014) 41:633–45. doi: 10.1016/j.immuni.2014.09.007
62. Goronzy JJ, Fulbright JW, Crowson CS, Poland GA, O'Fallon WM, Weyand CM. Value of immunological markers in predicting responsiveness to influenza vaccination in elderly individuals. *J Virol.* (2001) 75:12182–7. doi: 10.1128/JVI.75.24.12182-12187.2001
63. Saurwein-Teissl M, Lung TL, Marx F, Gschösser C, Asch E, Blasko I, et al. Lack of antibody production following immunization in old age: association with CD8(+)CD28(-) T cell clonal expansions and an imbalance in the production of Th1 and Th2 cytokines. *J Immunol.* (2002) 168:5893–9. doi: 10.4049/jimmunol.168.11.5893
64. Betjes MGH, Litjens NHR. High numbers of differentiated CD28null CD8+ T cells are associated with a lowered risk for late rejection and graft loss after kidney transplantation. *PLoS ONE.* (2020) 15:e0228096. doi: 10.1371/journal.pone.0228096
65. Dedeoglu B, Meijers RW, Klepper M, Hesselink DA, Baan CC, Litjens NH, et al. Loss of CD28 on peripheral T cells decreases the risk for early acute rejection after kidney transplantation. *PLoS ONE.* (2016) 11:e0150826. doi: 10.1371/journal.pone.0150826
66. Morris AB, Farley CR, Pinelli DF, Adams LE, Cragg MS, Boss JM, et al. Signaling through the inhibitory Fc receptor FcγRIIB induces CD8(+) T cell apoptosis to limit t cell immunity. *Immunity.* (2020) 52:136–50. e6. doi: 10.1016/j.immuni.2019.12.006
67. Betjes MG. Clinical consequences of circulating CD28-negative T cells for solid organ transplantation. *Transpl Int.* (2016) 29:274–84. doi: 10.1111/tri.12658
68. Rocamora-Reverte L, Melzer FL, Würzner R, Weinberger B. The complex role of regulatory T cells in immunity and aging. *Front Immunol.* (2020) 11:616949. doi: 10.3389/fimmu.2020.616949
69. Litjens NH, Boer K, Zuijderwijk JM, Klepper M, Peeters AM, Verschoor W, et al. Natural regulatory T cells from patients with end-stage renal disease can be used for large-scale generation of highly suppressive alloantigen-specific Tregs. *Kidney Int.* (2017) 91:1203–13. doi: 10.1016/j.kint.2016.09.043
70. Zook EC, Krishack PA, Zhang S, Zeleznik-Le NJ, Firulli AB, Witte PL, et al. Overexpression of Foxn1 attenuates age-associated thymic involution and prevents the expansion of peripheral CD4 memory T cells. *Blood.* (2011) 118:5723–31. doi: 10.1182/blood-2011-03-342097
71. Sato K, Kato A, Sekai M, Hamazaki Y, Minato N. Physiologic thymic involution underlies age-dependent accumulation of senescence-associated CD4(+) T cells. *J Immunol.* (2017) 199:138–48. doi: 10.4049/jimmunol.1602005
72. Sauce D, Larsen M, Fastenackels S, Duperrier A, Keller M, Grubeck-Loebenstein B, et al. Evidence of premature immune aging in patients thymectomized during early childhood. *J Clin Invest.* (2009) 119:3070–8. doi: 10.1172/JCI39269
73. Litjens NH, de Wit EA, Betjes MG. Differential effects of age, cytomegalovirus-seropositivity and end-stage renal disease (ESRD) on circulating T lymphocyte subsets. *Immun Ageing.* (2011) 8:2. doi: 10.1186/1742-4933-8-2
74. Huang L, Litjens NHR, Kannegieter NM, Klepper M, Baan CC, Betjes MGH. pERK-dependent defective TCR-mediated activation of CD4(+) T cells in end-stage renal disease patients. *Immun Ageing.* (2017) 14:14. doi: 10.1186/s12979-017-0096-1
75. Li G, Yu M, Lee WW, Tsang M, Krishnan E, Weyand CM, et al. Decline in miR-181a expression with age impairs T cell receptor sensitivity by increasing DUSP6 activity. *Nat Med.* (2012) 18:1518–24. doi: 10.1038/nm.2963
76. Grasselli G, Zangrillo A, Zanella A, Antonelli M, Cabrini L, Castelli A, et al. Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the lombardy region, italy. *JAMA.* (2020) 323:1574–81. doi: 10.1001/jama.2020.5394
77. Council E-E, Group EW. Chronic kidney disease is a key risk factor for severe COVID-19: a call to action by the ERA-EDTA. *Nephrol Dial Transplant.* (2021) 36:87–94. doi: 10.1093/ndt/gfaa314
78. Rydyznski Moderbacher C, Ramirez SI, Dan JM, Grifoni A, Hastie KM, Weiskopf D, et al. Antigen-specific adaptive immunity to SARS-CoV-2 in acute COVID-19 and associations with age and disease severity. *Cell.* (2020) 183:996–1012. e19. doi: 10.1016/j.cell.2020.09.038
79. García LF. Immune response, inflammation, and the clinical spectrum of COVID-19. *Front Immunol.* (2020) 11:1441. doi: 10.3389/fimmu.2020.01441
80. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with (2019) novel coronavirus in Wuhan, China. *Lancet.* (2020) 395:497–506. doi: 10.1016/S0140-6736(20)30183-5
81. Vabret N, Britton GJ, Gruber C, Hegde S, Kim J, Kuksin M, et al. Immunology of COVID-19: current state of the science. *Immunity.* (2020) 52:910–41.
82. Hoagland DA, Møller R, Uhl SA, Oishi K, Frere J, Golynker I, et al. Leveraging the antiviral type I interferon system as a first line of defense against SARS-CoV-2 pathogenicity. *Immunity.* (2021) 54:557–70. doi: 10.1016/j.immuni.2021.01.017
83. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. *J Clin Invest.* (2020) 130:2620–9. doi: 10.1172/JCI137244
84. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al. Dysregulation of immune response in patients with coronavirus 2019 (COVID-19) in Wuhan, China. *Clin Infect Dis.* (2020) 71:762–8. doi: 10.1093/cid/ciaa248
85. Wang F, Nie J, Wang H, Zhao Q, Xiong Y, Deng L, et al. Characteristics of peripheral lymphocyte subset alteration in COVID-19 pneumonia. *J Infect Dis.* (2020) 221:1762–9. doi: 10.1093/infdis/jiaa150
86. Zheng M, Gao Y, Wang G, Song G, Liu S, Sun D, et al. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. *Cell Mol Immunol.* (2020) 17:533–5. doi: 10.1038/s41423-020-0402-2
87. Wan S, Yi Q, Fan S, Lv J, Zhang X, Guo L, et al. Relationships among lymphocyte subsets, cytokines, and the pulmonary inflammation index in coronavirus (COVID-19) infected patients. *Br J Haematol.* (2020) 189:428–37. doi: 10.1111/bjh.16659
88. De Biasi S, Meschiari M, Gibellini L, Bellinazzi C, Borella R, Fidanza L, et al. Marked T cell activation, senescence, exhaustion and skewing towards TH17 in patients with COVID-19 pneumonia. *Nat Commun.* (2020) 11:3434. doi: 10.1038/s41467-020-17292-4
89. Liu J, Li S, Liu J, Liang B, Wang X, Wang H, et al. Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. *EBioMedicine.* (2020) 55:102763. doi: 10.1016/j.ebiom.2020.102763
90. Betjes MG, de Wit EE, Weimar W, Litjens NH. Circulating pro-inflammatory CD4posCD28null T cells are independently associated with cardiovascular disease in ESRD patients. *Nephrol Dial Transplant.* (2010) 25:3640–6. doi: 10.1093/ndt/gfq203
91. Meijers RW, Litjens NH, Hesselink DA, Langerak AW, Baan CC, Betjes MG. Primary cytomegalovirus infection significantly impacts circulating T cells in kidney transplant recipients. *Am J Transplant.* (2015) 15:3143–56. doi: 10.1111/ajt.13396
92. Vescovini R, Biasini C, Fagnoni FF, Telera AR, Zanlari L, Pedrazzoni M, et al. Massive load of functional effector CD4+ and CD8+ T cells against cytomegalovirus in very old subjects. *J Immunol.* (2007) 179:4283–91. doi: 10.4049/jimmunol.179.6.4283
93. Redeker A, Remmerswaal EBM, van der Gracht ETI, Welten SPM, Hollt T, Koning F, et al. The contribution of cytomegalovirus infection to immune senescence is set by the infectious dose. *Front Immunol.* (2017) 8:1953. doi: 10.3389/fimmu.2017.01953
94. Betjes MG, Huisman M, Weimar W, Litjens NH. Expansion of cytolytic CD4+CD28- T cells in end-stage renal disease. *Kidney Int.* (2008) 74:760–7. doi: 10.1038/ki.2008.301
95. Huang L, Langerak AW, Baan CC, Litjens NH, Betjes MG. Latency for cytomegalovirus impacts T cell ageing significantly in elderly end-stage renal disease patients. *Clin Exp Immunol.* (2016) 186:239–48. doi: 10.1111/cei.12846
96. Zhang X, Feng X, Cai W, Liu T, Liang Z, Sun Y, et al. Chemokine CX3CL1 and its receptor CX3CR1 are associated with human atherosclerotic lesion volnerability. *Thromb Res.* (2015) 135:1147–53. doi: 10.1016/j.thromres.2015.03.020
97. Betjes MG, Meijers RW, de Wit LE, Litjens NH. A killer on the road: circulating CD4(+)CD28null T cells as cardiovascular risk factor in ESRD patients. *J Nephrol.* (2012) 25:183–91. doi: 10.5301/jn.5000057

98. Betjes MG, Weimar W, Litjens NH. Circulating CD4(+)CD28null T cells may increase the risk of an atherosclerotic vascular event shortly after kidney transplantation. *J Transplant.* (2013) 2013:841430. doi: 10.1155/2013/841430
99. Xiong Y, Liu Y, Cao L, Wang D, Guo M, Jiang A, et al. Transcriptomic characteristics of bronchoalveolar lavage fluid and peripheral blood mononuclear cells in COVID-19 patients. *Emerg Microbes Infect.* (2020) 9:761–70. doi: 10.1080/22221751.2020.1747363
100. Zhou Z, Ren L, Zhang L, Zhong J, Xiao Y, Jia Z, et al. Heightened Innate Immune Responses in the Respiratory Tract of COVID-19 Patients. Cell Host Microbe. (2020) 27:883–90. e2 doi: 10.1016/j.chom.2020.04.017
101. Ronit A, Berg RMG, Bay JT, Haugaard AK, Ahlström MG, Burgdorf KS, et al. Compartmental immunophenotyping in COVID-19 ARDS: a case series. *J Allergy Clin Immunol.* (2021) 147:81–91. doi: 10.1016/j.jaci.2020.09.009
102. Liao M, Liu Y, Yuan J, Wen Y, Xu G, Zhao J, et al. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. *Nat Med.* (2020) 26:842–4. doi: 10.1038/s41591-020-0901-9
103. Group WHOREAfC-TW, Sterne JAC, Murthy S, Diaz JV, Slutsky AS, Villar J, et al. Association between administration of systemic corticosteroids and mortality among critically ill patients with covid-19: a meta-analysis. *JAMA.* (2020) 324:1330–41.
104. Belli LS, Fondevila C, Cortesi PA, Conti S, Karam V, Adam R, et al. Protective role of tacrolimus, deleterious role of age and comorbidities in liver transplant recipients with Covid-19: results from the ELITA/ELTR multi-center European study. *Gastroenterology.* (2020) 160:1151–63. doi: 10.1053/j.gastro.2020.11.045
105. Betjes MG. Uremia-associated ageing of the thymus and adaptive immune responses. *Toxins.* (2020) 12:224. doi: 10.3390/toxins12040224
106. Thomas R, Wang W, Su DM. Contributions of age-related thymic involution to immunosenescence and inflammaging. *Immun Ageing.* (2020) 17:2. doi: 10.1186/s12979-020-0173-8
107. Cheng L, Guo J, Sun L, Fu J, Barnes PF, Metzger D, et al. Postnatal tissue-specific disruption of transcription factor FoxN1 triggers acute thymic atrophy. *J Biol Chem.* (2010) 285:5836–47. doi: 10.1074/jbc.M109.072124
108. Sun L, Guo J, Brown R, Amagai T, Zhao Y, Su DM. Declining expression of a single epithelial cell-autonomous gene accelerates age-related thymic involution. *Aging Cell.* (2010) 9:347–57. doi: 10.1111/j.1474-9726.2010.00559.x
109. Chu YW, Memon SA, Sharrow SO, Hakim FT, Eckhaus M, Lucas PJ, et al. Exogenous IL-7 increases recent thymic emigrants in peripheral lymphoid tissue without enhanced thymic function. *Blood.* (2004) 104:1110–9. doi: 10.1182/blood-2003-10-3635
110. Henson SM, Snelgrove R, Hussell T, Wells DJ, Aspinall R. An IL-7 fusion protein that shows increased thymopoietic ability. *J Immunol.* (2005) 175:4112–8. doi: 10.4049/jimmunol.175.6.4112
111. Meijers RW, Litjens NH, de Wit EA, Langerak AW, Baan CC, Betjes MG. Uremia-associated immunological aging is stably imprinted in the T-cell system and not reversed by kidney transplantation. *Transpl Int.* (2014) 27:1272–84. doi: 10.1111/tri.12416
112. Yang H, Youm YH, Vandanmagsar B, Rood J, Kumar KG, Butler AA, et al. Obesity accelerates thymic aging. *Blood.* (2009) 114:3803–12. doi: 10.1182/blood-2009-03-213595
113. Jongbloed F, Meijers RWJ, JNM IJ, Klaassen RA, Dolle MET, van den Berg S, et al. Effects of bariatric surgery on telomere length and T-cell aging. *Int J Obes.* (2019) 43:2189–99. doi: 10.1038/s41366-019-0351-y
114. Duggal NA, Niemiro G, Harridge SDR, Simpson RJ, Lord JM. Can physical activity ameliorate immunosenescence and thereby reduce age-related multi-morbidity? *Nat Rev Immunol.* (2019) 19:563–72. doi: 10.1038/s41577-019-0177-9
115. Duggal NA, Pollock RD, Lazarus NR, Harridge S, Lord JM. Major features of immunesenescence, including reduced thymic output, are ameliorated by high levels of physical activity in adulthood. *Aging Cell.* (2018) 17:e12750. doi: 10.1111/acel.12750

Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

REVIEW
published: 03 September 2021
doi: 10.3389/fmed.2021.720402

End-Stage Renal Disease-Related Accelerated Immune Senescence: Is Rejuvenation of the Immune System a Therapeutic Goal?

Didier Ducloux[1,2,3,4]*, *Mathieu Legendre*[1,2], *Jamal Bamoulid*[1,2,3,4], *Philippe Saas*[1,2,3,5], *Cécile Courivaud*[1,2,3,4] and *Thomas Crepin*[1,4]

[1] Inserm, UMR1098, Federation Hospitalo-Universitaire INCREASE, Besançon, France, [2] University Bourgogne Franche-Comté, Faculté de Médecine et de Pharmacie, LabEx LipSTIC, Besançon, France, [3] Structure Fédérative de Recherche, SFR FED4234, Besançon, France, [4] CHU Besançon, Department of Nephrology, Dialysis, and Renal Transplantation, Besançon, France, [5] EFS Bourgogne Franche-Comté, Plateforme de Biomonitoring, CIC 1431/UMR1098, Besançon, France

OPEN ACCESS

Edited by:
Maria J. Stangou,
Aristotle University of Thessaloniki, Greece

Reviewed by:
Konstantinos Bantis,
Hippokration General Hospital, Thessaloniki, Greece
Xiaohong Chen,
Fudan University, China

***Correspondence:**
Didier Ducloux
dducloux@chu-besancon.fr
orcid.org/0000-0003-3841-1211

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: 04 June 2021
Accepted: 17 August 2021
Published: 03 September 2021

Citation:
Ducloux D, Legendre M, Bamoulid J, Saas P, Courivaud C and Crepin T (2021) End-Stage Renal Disease-Related Accelerated Immune Senescence: Is Rejuvenation of the Immune System a Therapeutic Goal? Front. Med. 8:720402. doi: 10.3389/fmed.2021.720402

End-stage renal disease (ESRD) patients exhibit clinical features of premature ageing, including frailty, cardiovascular disease, and muscle wasting. Accelerated ageing also concerns the immune system. Patients with ESRD have both immune senescence and chronic inflammation that are resumed in the so-called inflammaging syndrome. Immune senescence is particularly characterised by premature loss of thymic function that is associated with hyporesponsiveness to vaccines, susceptibility to infections, and death. ESRD-related chronic inflammation has multiple causes and participates to accelerated cardiovascular disease. Although, both characterisation of immune senescence and its consequences are relatively well-known, mechanisms are more uncertain. However, prevention of immune senescence/inflammation or/and rejuvenation of the immune system are major goal to ameliorate clinical outcomes of ESRD patients.

Keywords: immune senescence, thymus, inflammaging, end-stage renal disease, kidney transplantation

INTRODUCTION

Patients with end-stage renal disease (ESRD) are especially prone to infection (1). Furthermore, concordant data also report that immune responses against vaccines are considerably reduced in this population (2). Concomitantly, ESRD patients exhibit aseptic low-grade inflammation (3, 4). These clinical features are close to those observed in elderly and suggest that inflammaging associating both premature senescence of the immune system and inflammation is a key part of the ESRD-related immune phenotype. What is more, some convincing studies established evidences of accelerated immune senescence in chronic kidney disease and dialysis patients compared to the general population (5–7).

In this review, we analyze recent knowledge on ESRD-associated accelerated immune ageing with a special focus on thymus involution. In addition, we speculate on therapeutic tools likely to prevent or reverse these immune alterations.

IMMUNE SENESCENCE IN AGEING

The term immune senescence clusters all the changes that occur in the immune system during ageing. Although this process mainly affects T lymphocytes, all aspects of innate and adaptive immunity are concerned. Recently, immune ageing has been suggested to be more appropriate to design all immune changes associated with ageing. Indeed, the ageing of the immune system is a more general concept including two different processes. The first one is what specifically refers to immune senescence, which is mainly linked to age-dependent thymic involution leading to reduced immune repertoire diversity and compounded oligo-clonal increase in memory immune cells. Sensitivity to infections, reduced vaccine immunity, and defect in tumour clearance observed in elderly are thought to be at least in part linked to these immune alterations. Immune senescence in T cells is sometimes called cellular exhaustion even if the two phenomenon are not exactly identical. Exhausted T cells are defined by the loss of CD28 and the concomitant expression of Tim-3 and PD-1 (8). The second characteristics of aged immunity is inflammaging. Old age is associated with low-grade systemic inflammation. Chronic innate immune activation, pro-inflammatory cytokine profile secretion, and age-induced accumulation of self-reactive T cells contribute to age-related inflammation. Inflammaging is supposed to explain some degenerative disease associated with ageing. The term "Inflammaging" is frequently proposed to include these two aspects.

Immune Senescence: A Pivotal Role of Thymic Involution

T cell immune senescence is mainly linked to physiologic thymic involution. The thymus mainly serves to the development of a large but self-tolerant T cell repertoire. Briefly, multipotent hematopoietic stem cells (HSC) differentiate into common lymphoid or myeloid progenitors. T lymphoids precursors go to the thymus where they undergo several stages of maturation resulting in the formation of naive T lymphocytes called recent thymic emigrant (RTE) (9). These cells present a diversified polyclonal T cell receptor (TCR). Central tolerance occurs in the thymus *via* two mechanisms. The first one is thymocyte negative selection. This step consists in deletion of most of self-auto-reactive T cells *via* apoptosis (9). The second concerns the generation of CD4 single positive FoxP3+ regulatory T cells, which can eliminate auto-reactive T cells having escaped to negative selection (9).

The ability to generate RTE in the thymus declines with age. Thymic involution consists in reduction of both thymic size and thymocyte number and reorganisation of thymic ultrastructure. Soon after birth, functional tissue begins to be substituted by fat (10). After 50 years, there is almost no output of naïve T cells. The frequency of naïve T cells greatly diminishes both in periphery and in lymphoid organ, especially for CD8+ T cells. Nevertheless, homeostatic proliferation of previously generated naïve T cells enables to maintain a broad and diverse pool of naïve T cells, especially for CD4+ T cells. Continuous involution of the thymus with age finally causes a decrease in the thymic output of naïve T cells and subsequently a reduction of the peripheral TCR repertoire.

Two non-exclusive mechanisms account for thymus involution. The first one is mainly based on a reduced production of HSC. Indeed, self-renewal of HSC diminishes with age and tends to favour myeloid lineage (11, 12). Reduction in HSC production and switch toward myeloid lineage would both contribute to decrease the output of common lymphoid progenitors (13, 14). In addition, aged hematopoietic stem cells have less lymphoid differentiation potential (15). The second one depends on age-related reduction in stromal niches of the bone marrow () and thymus (16, 17). Recent studies mainly plead for the latter hypothesis. Stroma cells in the thymus are mainly thymic epithelial cells (TECs) (18). Convincing data show that age-associated thymic involution is dependent on TEC transcription factors involved in TEC homeostasis, such as Forkhead box N1 (19). Indeed, FOXN1 is essential for thymus development and thymocyte formation (19). A null mutation in the FOXN1 gene defines the "null mice" which phenotype is characterised, amongst others, by the absence of thymus and T cells (20). Reduction in thymic FOXN1 expression is observed as one the first step of thymic involution in aged individuals (21). Conditional KO mice studies have considerably explained the causal role of FOXN1 in thymus involution. LoxP-floxed-FoxN1 mouse with the ubiquitous CreER(T) transgene have a low dose of spontaneous activation and exhibit progressive loss of FOXN1 (22). Progressive loss of FOXN1 is associated with accelerated thymic involution (22). Finally, intra-thymic supplementation in FOXN1-cDNA partially reverses thymic involution and restores peripheral CD4+ T cell population (22).

The decrease in T cell production is compensated by homeostatic expansion of existing peripheral T cells occurs. This leads to an increased proportion of memory T cells and reduction in the diversity of TCR repertoire (23). Accumulation of memory T cells is mainly due to life-long exposure to chronic antigen stimulation by pathogens. The most important is cytomegalovirus. However, expansion of CD8+ T cells is only observed in CMV-exposed old patients (24).

Inflammaging

Somatic cellular senescence is defined by the permanent arrest of cell cycle accompanied by lack of proliferation, expression of anti-proliferative markers, and shortening of telomeres (25). This biological process is likely to be protective against cancer transformation (26).

Accumulation of somatic senescent cells contribute on one hand to organ dysfunction, and on the other hand to inflammation through induction of somatic cell senescence-associated secretory phenotype (SASP). Immune senescence favours increased production of SASP (27) due to decreased chemotaxis of immune cells toward somatic senescent cells and reduced phagocytosis by neutrophils and macrophages (28–31).

Many other mechanisms contribute to inflammation in ageing. Chronic viral infections, especially with CMV, induce low level of cytokines production (32).

Moreover, involution of the thymus is accompanied by a decrease ability to negatively select self-auto-reactive T cells,

which explain propension to certain autoimmune diseases in the elderly (33). Paradoxically, peripheral Treg cells accumulate during ageing. Thomas et al. (34) developed a mock-self-antigen chimaera mouse model, in which membrane-bound ovalbumin transgenic mice, carrying a FOXN1-floxed gene for induction of conditional thymic atrophy, received ovalbumin-specific T cell receptor transgenic progenitor cells. The authors showed that a decreased number of ovalbumin-specific tTreg and pTreg, but not polyclonal Treg cells in chimeric mice with thymus atrophy. The ovalbumin-specific pTreg had less suppressive activity and a lower expression of FoxP3. This suggest that although generation of polyclonal pan-Treg is not affected by thymus involution, certain specific Treg clones may have aberrant agonist selection contributing to age-related chronic inflammation.

Thus, immune ageing is characterised by both immune deficiency (immune senescence driven by thymus involution) and inflammation leading to the concept of inflammaging.

IMMUNE AGEING IN END-STAGE RENAL DISEASE

Chronic kidney disease phenotype is very similar to premature ageing. Frailty, osteoporosis, muscle wasting, and cardiovascular disease occur at younger age in CKD patients. Many factors such as oxidative stress, accumulation of uremic toxins, and inflammation are supposed to contribute to accelerated ageing (35). The immune system undergoes a similar premature ageing. Indeed, peripheral blood mononuclear cell relative telomere length is shorter in CKD patients as compared to healthy individuals (5). Furthermore, ESRD patients frequently exhibit T cell lymphopenia (6) and concomitantly have both a marked susceptibility for infections and a decreased response to vaccines suggesting a T cell immune defect (7). Finally, ESRD patients exhibit a low-grade inflammation status (36). This association is typical of the "inflammaging" state observed in elderly.

Premature thymic involution is a key component of ESRD-associated immune senescence. Others and we reported that thymic output decreased with progression of CKD. Thymic output is comparable between 40-year-old uremic patients and 80 year-old non-uremic patients (5). Our group recently reported that, in ESRD patients, low thymic output was predictive of severe infections (5). The decrease in RTE could be the result of a reduction in the thymic output of naïve T cells and/or of a reduction in homeostatic proliferation. Premature loss of thymic function is likely to explain the decrease in naïve T cells in young patients with ESRD. Indeed, decreased CD4 naïve T cells percentage is also observed in paediatric CKD patients (37). Moreover, concordant data in animals suggest that acute renal failure accelerates thymus involution (38, 39).

However, there are few data documenting potential causes for premature thymic involution during chronic kidney disease. Chronic inflammation is likely to markedly contribute to immune ageing. Of note, a recent study shows that CRP levels inversely correlates with naïve T cells in haemodialysis patients suggesting either that inflammation and immune senescence evolve in parallel or that one is driving the other one (40). Activation of innate immunity, characterised by monocyte activation and overproduction of inflammatory cytokines such as Il-6, is a key feature of the CKD immune system (4, 41, 42). Thus, Jurk et al. (43) reported that knockout of the nfkb1 subunit of the transcription factor NF-κB induces chronic low-grade inflammation that leads to premature ageing in mice. Treating reversible source of inflammation is obviously a goal in CKD patients and such strategy may reduce premature ageing.

Main mechanisms of premature immune ageing are summarised in **Figure 1**.

IMMUNE REJUVENATION: FACTS AND PERSPECTIVES IN CKD

Immune senescence has deleterious consequences. Susceptibility to infection, premature cardiovascular disease, and increased cancer incidence are some of the most frequent and serious. A number of measures, from the simplest to the more complex, may be susceptible to reverse immune senescence, especially premature thymic involution (**Figure 2**).

PHYSICAL ACTIVITY

The impact of physical activity in maintaining thymic activity must not be neglected. It is one of the rare therapeutic strategies with consistent results in both animal and human studies (44).

In an immunological ageing mouse model, 4 weeks of free-wheel running increased naïve T lymphocytes and reduced effector ratio of cytotoxic T lymphocytes (45). Concordant data also exist in humans. Comparing adults (55–79 years) who had intensive sportive practise (cycling), age-matched adults and young sedentary adults, Duggal et al. (46) observed increased frequency of naïve T cells and RTE in cyclists. Sportsmen had also higher levels of IL-7 and lower levels of IL-6. By contrast, CD28-CD57+CD8+ T cell frequencies did not differ between the three groups. Even more powerful are the evidence that sustained physical activity in elderly improve immune responses against influenza vaccine (47, 48).

Skeletal muscles express and secrete different cytokines, also called myokines. Among them, IL-7 and IL-15 are released during exercise (49, 50). IL-6 is also released by muscle during exercise. Nevertheless, whereas IL-6 secreted through NF-KB signalling is pro-inflammatory, IL-6 produced by muscles is dependent on JUN N-terminal kinase and activator protein 1 signalling and exhibits anti-inflammatory properties (51, 52). Proof of concept is supported by experiments showing that both exercise and IL-6 infusion suppress inflammation induced by endotoxin injection (53). Modulation of cytokine secretion by muscles during exercise are likely to explain the link between physical activity and thymopoiesis.

Physical activity is often reduced in CKD patients. Sedentary life, socio-economics conditions, comorbidities, and uremia-related asthenia contribute to the reduced physical activity. Although a large number of studies reported the beneficial effects of exercise in CKD patients, no data are available concerning the potential consequences on immune status. However, other

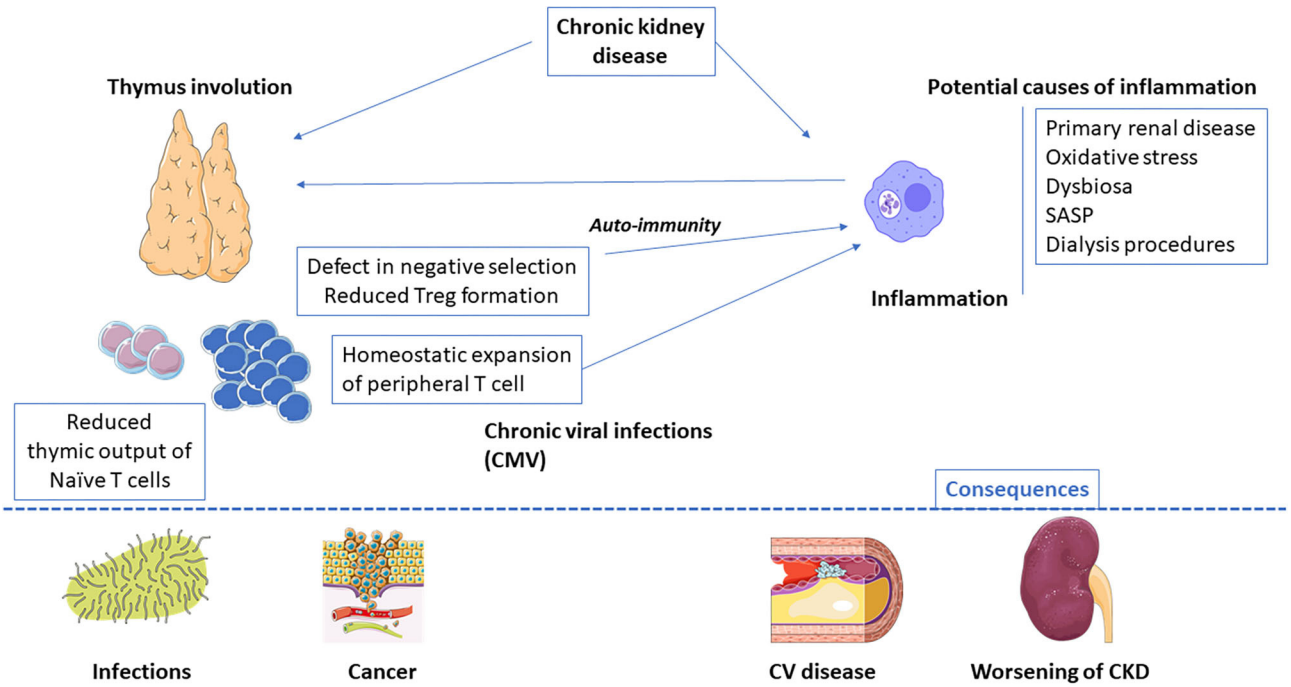


FIGURE 1 | End-stage renal disease-related inflammaging: main causes and potential consequences. CMV, Cytomegalovirus; SASP, senescence-associated secretory phenotype.

benefits of physical exercise in ESRD patients have been largely reported and physical rehabilitation programs should be encouraged in these patients. Further studies should analyze whether physical activity may at least in part reverse or prevent thymic involution and inflammation.

HORMONES

Many hormonal pathways play a role in thymic physiology. However, most of them are impaired during chronic renal failure.

IGF-1-GH Pathway

The IGF-1–GH pathway interferes with many aspects of thymus biology. TECs express GH receptors (54) and IGF is expressed in the thymus (55). Growth hormone supplementation increases thymic cytokine production and T cell progenitor recruitment into the thymus and can reverse thymic involution (56–58). Hansen et al. (59) reported that treatment with rhGH increased thymus size, T cell receptor excision circles (TREC) frequency, and total TREC content in CD4 T cells in HIV-infected patients. GH withdrawal in patients receiving GH treatment is followed by decreased thymic output and intra-thymic T cell proliferation (60).

The IGF-1-GH axis is profoundly altered in dialysis patients. ESRF patients have increased GH secretion, but normal IGF-1 concentrations, indicating GH resistance (61, 62). The resistant state is related to alterations at several levels of GH/IGF-1 axis, GH signalling, and IGF-1 action (63, 64). Several studies reported that GH administration might increase IGF-1 levels in dialysis patients as in healthy subjects (65). Moreover, large studies confirmed the safety of long-term administration of GH in dialysis patients (66).

All these data suggest that GH may be a therapeutic hope to reverse thymopoiesis defect in ESRD patients.

Sex Hormones

The effects of sex hormones on thymus are well-known. A number of studies demonstrated that sex steroid ablation delay or reverse thymus involution in both animals and humans (67, 68). Sex steroids inhibit TEC expression of Notch ligand Delta-like 4 that promotes T cell differentiation and development (69).

Surgical castration is obviously not a therapeutic option in humans, but LHRH analogues use is also associated with thymic rejuvenation (70). Leuprolide desensitises LHRH receptors and reduce the release of LH and FSH. Goldberg et al. (71) showed that Leuprolide enhances T cell reconstitution following allogeneic bone marrow transplantation in mice. Similar data have been obtained in non-human primates (72). Leuprolide induces thymic rejuvenation in aged male baboons (72). Castration by Leuprolide is reversible and, due to the use of LHRH agonists in a variety of human diseases, safety, pharmacokinetics and efficacy are well-known.

Nevertheless, some studies also suggest that castration-induced thymic rejuvenation is only transient and potentially hazardous. Indeed, sex hormones deprivation favours self-reactivity (73, 74). Concordant with this concern, castration decreased CD4+CD25+ Treg and increased natural (NK) cells in humans (75). Moreover, androgens increase autoimmune

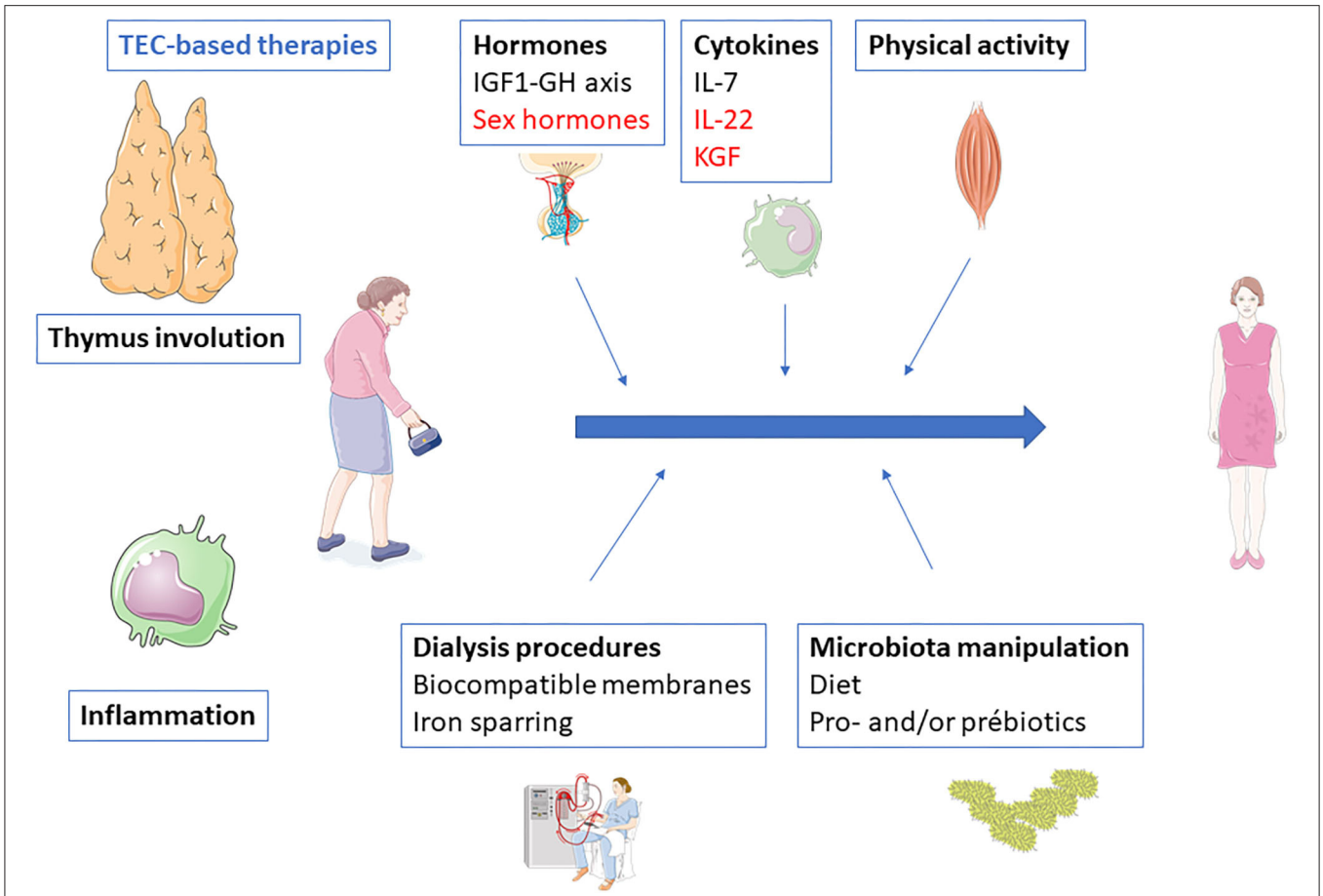


FIGURE 2 | Potential therapeutic interventions to prevent or reverse end-stage renal disease-related inflammaging. Black, interventions with evidences; Blue, interventions without clinical evidence but with strong background and potentiality; Red, interventions with evidences of inefficacy.

regulator (AIRE) expression in mTEC and therefore enhance negative thymocyte selection while estrogens have opposite effects (76).

Despite some former results, the use of chemical castration to enhance thymic rejuvenation is consequently not a safe option.

CYTOKINES

IL-7

IL-7 is produced by both thymic stromal cells and bone marrow. IL-7 mediates lymphopoiesis of both T and B cells, and in the thymus, promotes proliferation, differentiation, and survival of thymocytes (77). IL-7 signals through its receptor IL-7R (78). Loss of function mutations in IL-7R leads to severe combined immunodeficiency (SCID) (78).

Administration of IL-7 in mice expand both naïve and memory CD4 and CD8 peripheral T cells (79).

RhIL-7 has been used in different clinical settings and constantly leads to increase circulating T cell populations, with more specific expansion of RTE, naïve T cells and central memory T cells (80–83). TCR repertoire diversification is also observed in rhIL-7 treated patients (84). The increase in both CD4+ and CD8+ T cell remain for months after the end of treatment by rhIL-7 (85).

IL-7 concentrations have been found to be elevated in CKD (86) suggesting a possible relative resistance to this cytokine. Nevertheless, to date, there is no study assessing the effects of rhIL-7 in lymphopenic CKD patients. Our group recently begun a phase II study (INDIA Study NCT…) using rhIL-7 to reverse thymic involution in ESRD patients on dialysis.

IL-22

Interleukin-22, also called IL-10-related T cell-derived inducible factor (IL-TIF) (87), is a member of the IL-10 family, including IL-19, IL-20, IL-24, IL-26, IL-28, and IL-29. IL-22R1 determines the cellular sensitivity toward IL-22. This receptor is restricted to specific cell types and is absent on immune cells (88). Il-22 interacts with IL-2R on the surface of TEC and allows both survival and proliferation of thymocytes.

IL-22 administration to mice having received total body irradiation increases both thymocytes and TEC recovery (89). Similar observations have been done after murine allogeneic hematopoietic cell transplant (90). IL-22 increases the number of TEC *via* a stat3-dependent signalling (91). More recently, it

was shown that, after allogeneic hematopoietic transplantation, plasma IL-22 levels positively correlated with blood TREC levels (92).

Limitations in the therapeutic use of rhIL-22 are based on its dual effects, which strictly depend on the context. The pro-regenerative effects of IL-22 could be counterbalanced by its inflammatory and tumorigenic properties.

Keratinocyte Growth Factor

KGF belongs to the fibroblast growth factor family. This cytokine is involved in epithelial cell proliferation and differentiation in many tissues, including the thymus. KGF KO mice have impaired thymopoiesis and peripheral T-cell recovery after allogeneic bone marrow transplant (93). Moreover, KGF administration to mice enhance thymopoiesis and accelerate thymic recovery after irradiation (93, 94). In non-human primates, KGF enhances immune reconstitution after autologous hematopoietic progenitor cell transplantation (95, 96).

More recently, conflicting results made the benefits of KGF less clear. Coles et al. (97) reported on treatment with Palifermin (KGF) in patients having received alemtuzumab, a monoclonal anti-CD52 antibody, which induces profound and sustained T cell lymphopenia. Six months after treatment, individuals having received Palifermin had fewer naïve CD4+ T cells and sjTREC, leading to study discontinuation (97). Furthermore, in HIV-infected patients, Palifermin was not effective in either improving thymic function or rising circulating CD4+ T cells (98). Finally, Palifermin was associated with worse clinical outcomes in patients with acute respiratory distress syndrome (99).

All these results underline the difficulty to export results obtained in animal studies to humans and are to make cautious on KGF use.

FOXN1- AND TEC-BASED APPROACHES

Some studies tested whether TEC stem cell may help to restore thymic function.

In a mouse model, Kim et al. (100) reported that engraftment of young TEC allows thymic growth and increased T cell production. FOXN1-induced TEC from fibroblasts support CD4+ and CD8+ T cells development. Transplantation of such cells allows the formation of a complete thymus containing all the TEC subtypes required for T-cell differentiation (101). Another group recently confirmed the feasibility and relevance of such a strategy (102). Moreover, forced expression of FOXN1 in involuted thymus results in thymic regeneration with increased thymopoiesis and naïve T cell output (103). The structure of the regenerated thymus was very close to young thymus in terms of architecture and gene expression. These results suggests that up-regulation of FOXN1 is sufficient to reverse age-related thymic involution. Finally, recombinant FOXN1 protein fused with cell-penetrating peptides increased the number of TEC and enhanced thymopoiesis after hematopoietic stem cell transplantation in mice (104).

All together, these studies suggest that the FOXN1 axis research is a valuable strategy to reverse thymic involution. To date, there are no evaluation of FOXN1 expression during CKD.

MICROBIOTA

Microbiota interferes with the immune system lifelong and its dysregulation results in inflammation (105). After great variations during the neonatal and early life periods, more subtle changes occur in microbiota until middle age before final stabilisation (106). Nevertheless, age-related changes in intestinal functions, inflammation, and co-morbidities may contribute to dysbiosis (107).

Whether microbiota interferes with immune senescence is challenging because the relative part of microbiota and health status are difficult to isolate. Moreover, even when dysbiosis may favour inflammation, inflammation may also promote dysbiosis asking the question of which came first the chicken or the egg? Indeed, chronic inflammation is a potent driver of increased gut permeability and microbial dysbiosis. For instance, age-induced dysbiosis is reduced in TNF KO mice as compared with wild type (108). Moreover, some cytokines decrease expression of tight junction proteins favours gut permeability, bacterial translocation, and systemic inflammation (109).

There are scarce but convincing data suggesting that aged microbiota contributes to drive immune senescence. Young germ-free (GF) mice raised with aged mice exhibit an inflammatory profile characterised by elevated inflammatory cytokines and macrophage activation (108). This effect was not observed when young GF mice were co-housed with young mice (108). Fransen et al. (109) reported on the transfer of gut microbiota from conventional old mice to young GF mice. T cell activation occurs in young GF mice after transfer of microbiota. Inflammation was related to higher levels of Proteobacteria and lower levels of Akkermansia in old CV mice. Once again, these alterations in immune status were not observed after transfer of microbiota from young conventional mice.

Short-chain fatty acid levels decreased in elderly. Yet, SCFA lead to increase Treg cell differentiation (110). SCFA supplementation, namely butyrate, suppresses arthritis in mice by a Breg-dependent mechanism (111). Precisely, Butyrate increases the levels of 5-HIAA (5-hydroxyindole-3-acetic acid) which activates the ary-hydrocarbon receptor, a transcriptional marker for Breg function (111).

Administration of high dose probiotics in elderly subjects enhanced CD8+CD25+ T cells and NK cells while low dose increased CD4+CD25+ and B lymphocytes (112). A more recent study reported that a probiotic mixture increased naive and regulatory T cells and decreased memory T cells (113).

Finally, best evidence of interactions between microbiota and immune senescence come from studies reporting better vaccine responses against influenza after treatment with pre- or probiotics. Akatsu et al. (114) performed a randomised study in elderly receiving enteral tube feeding. Patients received either a placebo or Bifidobacterium longum BB536. After influenza vaccine, patients having received BB536 exhibited higher levels of anti-H1N1 antibodies. Boge et al. (115) also showed increased response to influenza vaccination in elderly following prolonged administration of a probiotic. Other recent studies suggest that probiotics and prebiotics are effective to improve seroconversion and seroprotection after influenza vaccines (116, 117).

Dysbiosis is a hallmark of chronic kidney disease (118). Accumulation of uremic toxins in CKD leads to an insuatable excretion of urea, uric acid, and oxalates in the intestinal lumen (119). The enrichment in uremic toxins cause substantial modifications in gut physiology mainly an increased in permeability and in microbiota with an increase in uricase and urease-producing bacteria (120). Proteolytic fermentation leads to the formation of different uremic toxins such as p-cresyl sulphate and indoxyl-sulphate potentially aggravating the uremic status (120). Overgrowth of Bacteroidetes, Firmicutes, Ruminococcaceae and clostridia together with low abundance of Lactobacilli, Prevotellae, and bifidobacterium species depict main characteristics of intestinal microbiota of CKD patients compared to healthy subjects (121). Dysbiosis and increased gut permeability take account for bacterial translocation and inflammation (122). More recently, we reported (123) that the proportion of the inflammatory 14-carbon chain lipid A-LPS was increased in ESRD patients compared to healthy volunteers. Conversely, proportion of anti-inflammatory 18-carbon chain lipid A-LPS was decreased. Moreover, sera with predominance of 14-carbon chain lipid A-LPS induced higher secretion of pro-inflammatory cytokines than those with predominance of 18-carbon chain lipid A-LPS. TLR4 or LPS antagonists decreased LPS-induced cytokine production by monocytes, demonstrating an LPS-specific effect. This suggests that septic inflammation observed in ESRD is at least in part related to a shift toward more inflammatory LPS subtypes from altered microbiota.

Different uremic toxins are generated in the intestine and contribute to inflammation in CKD patients (124). p-Cresol is a product of the bacteria metabolization of the aromatic amino acid tyrosine in the colon. Increased levels of p-Cresol in CKD patients correlate with the expansion of terminally differentiated CD8+ T cells (125). A recent randomised study reported that nutritional intervention based on very low protein diet modifies microbiota toward a potential anti-inflammatory profile and reduces p-Cresyl Sulphate (126). This suggests that dietary interventions may mitigate uremic syndrome and immune ageing through microbiota modulation.

KIDNEY TRANSPLANTATION

Successful kidney transplantation reverses renal failure and increases life expectancy. Contrasting effects of kidney transplantation have been observed on immune senescence.

Our group studied markers of immune senescence before and after kidney transplantation. In patients not having received polyclonal antithymocyte globulins (ATG), both T cell relative telomere length and telomerase activity increased after transplantation whereas they were not modified in ATG-treated patients (127). This suggests that renal function recovery may induce a partial reversion of immunesenescence. Nevertheless, Meijers et al. (128) did not observe such changes in T cell RTL after transplantation.

By contrast, concordant data exist to state that thymic output do not increase in non-ATG treated patients and decrease in those having received ATG (127, 128). *In vitro*, ATG binds to TEC and exerts a complement-independent, dose-dependent cytotoxicity (129). Nevertheless, Preville et al. (130) suggested that ATG could not enter into the thymus. Indeed, the authors observed a dose-dependent T cell depletion in spleen and lymph nodes but not in the thymus. However, these first results were not confirmed in a swine model in which lymphodepletion occurs in the thymus after administration of ATG (131). Alternatively, ATG may decrease lymphoid progenitors (127).

ESRD-associated CD8+ T cell expansion tends to marginally increase after transplantation mainly due to CMV reactivation (132). Nevertheless, inflammation measured by CRP or different proinflammatory cytokines substantially dropped after transplantation (122).

All together, these results suggest that kidney transplantation does not reverse ESRD-associated accelerated thymus involution. Whether this absence of effect is due to fixed immune changes or competitive effects of immunosuppressive drugs is difficult to ascertain.

DIALYSIS PROCEDURES

Dialysis procedure itself is a source of inflammation. Bioincompatible membranes, prosthetic vascular accesses, PD solution are potential sources of immune activation.

Peritoneal Dialysis vs. Haemodialysis

Whether PD results in systemic inflammation is not clear. Some studies reported that longer PD duration results in higher IL-6 concentrations (133, 134), but others did not observe any increase in IL-6 or CRP levels (135). By contrast, a burst in inflammation is well-described during HD procedure (136–138). Expression of TLR2 and TLR4 on monocytes from patients on haemodialysis is increased (139) whereas the expression of TLR4 has been reported to be reduced on monocytes in patients with CKD not receiving dialysis (140). Bioincompatible dialyzer induces intermittent activation of monocytes and up-regulation of TLR4. Accordingly, we observed higher inflammatory monocytes counts in patients on HD as compared to those on PD (141).

We also reported higher relative telomerase activity in PD patients (141). Of note, some cytokines released during haemodialysis session, such as IFN-α, may inhibit telomerase activity in hematopoietic cells (142, 143).

Finally, T cell exhaustion was more pronounced in HD patients, especially in those with previous exposure to CMV (141). Different mechanisms may explain this difference. Persistent low-grade inflammation in HD patients may contribute to immune responses to self-antigens and pathological ageing by promoting T cell exhaustion. Alternatively, repeated antigenic stimulation of T cells during haemodialysis session may cause enhanced proliferation and accelerated ageing compared to PD.

Dialysis Membranes Choice as a Modulator of Inflammation

Bio-incompatible membranes induce sustained activation of innate immunity. During a dialysis session, both neutrophils

and monocytes are recruited and activated. After activation, these cells release a number of pro-inflammatory cytokines and complement pathways activators (144). Simultaneously, their phagocytic functions are markedly altered (145, 146). Dialysis membranes also mediate complement activation (147, 148). These phenomena induce persistent pro-inflammatory and pro-coagulant states and partly explain the oxidative burst observed in ESRD patients. Although a direct effect of bio-incompatibility on adaptive immunity is more difficult to demonstrate, inter-connexions between innate and adaptive immunity may explain the consequences of bio-incompatibility on T cell functions (149).

A major challenge to reduce immune activation during dialysis is the development and use of more biocompatible membranes.

Membranes may be modified to reduce oxidative stress. For instance, vitamin E-coded dialyzers reduced indoleamine 2,3-dioxygenase-1 activity and nitric oxide formation (150). Of note, TEC are especially vulnerable to oxidative DNA damage. Thymic stromal deficiency in catalase induces thymic atrophy (151). Treatment with antioxidant can delay the onset of thymus involution (151).

Recently, median cut-off (MCO) membranes characterised by wider pores and more uniformity in pore size were developed. These membranes reduce uremic toxins at a greater degree (152). A randomised study showed that MCO significantly decrease the expression of TNF-α mRNA and IL-6 mRNA in PBMC compared to high-flux dialyzers (153). Polymethyl methacrylate (PMMA) membranes can remove large-weight molecular substances thanks to their adsorptive capacities (154). PMMA membranes seem to be associated with lower pre-dialysis values of IL-6 (155). Contrary to other dialyzers, PMMA membranes are able to clear sCD40 which accumulation in ESRD is associated with unresponsiveness to hepatitis B vaccine (156).

Even when dialysis membrane influence cytokines clearance, complement and coagulation activation, and removal of uremic toxins, a direct impact on immune senescence is not yet proven. However, there are, as described above, several mechanisms linking inflammation and immune ageing. Further studies should examine the effects of different membranes on adaptive immunity, vaccine responses, and clinical outcomes.

Iron Supplementation

Iron supplementation is widely used in HD (157). Intravenous iron administration induces oxidative stress (158). Iron overload is associated with shorter telomere length in ESRD patients (141). Association between iron overload and telomere length has been reported in different studies (159–161). Reduced telomere length is associated with mortality in dialysis patients (5). Excessive iron load enhances ferroptosis (162), which has an important role in sterile inflammatory conditions such as tissue acute injury, ischemic-reperfusion injury, and neurotoxicity.

CONCLUSION

Premature thymic involution and chronic inflammation greatly contribute to increased morbidity and mortality in CKD patients. Mechanisms are likely to be multiple and interlinked. Even when the quest to fountain of youth is a pipe dream, there are many scientific opportunities to prevent or to, at least in part, reverse CKD-related immune senescence. Further studies should precisely define most important pathways driving premature immune ageing in CKD patients and best therapeutic options to control them.

AUTHOR CONTRIBUTIONS

DD, ML, and TC wrote the paper. PS and JB corrected the proofs. All authors participate in the works supporting this review.

FUNDING

This work was supported by grants from the Fondation Transplantation, the PHRC 2005 and 2011 (to DD), the Fondation de France (Appel d'offre Maladies Cardiovasculaires 2007 #2007 001859 to PS), the DHOS/INSERM/INCa (Appel d'offre Recherche Translationnelle 2008 to DD and PS), and the APICHU 2010 (to JB), from the Agence Nationale de la Recherche (Labex LipSTIC, ANR-11-LABX-0021) and the Région de Franche-Comté (support to Labex LipSTIC to PS). JB, CC, and TC received financial support from the Fondation Transplantation (#ET-031211 and #ET-050320, respectively). This work was a part of the RIALTO (Research in Immunology of AtheroscLerosis after TransplantatiOn) program. This work was also sponsored by the Fédération hospitalo-universitaire INCREASE (INtegrated Centre for Research in Inflammatory DisEASEs).

REFERENCES

1. Li PK, Chow KM. Infectious complications in dialysis-epidemiology and outcomes. *Nat Rev Nephrol.* (2011) 8:77–88. doi: 10.1038/nrneph.2011.194
2. Krueger KM, Ison MG, Ghossein C. Practical guide to vaccination in all stages of CKD, including patients treated by dialysis or kidney transplantation. *Am J Kidney Dis.* (2020) 75:417–25. doi: 10.1053/j.ajkd.2019.06.014
3. Ducloux D, Bresson-Vautrin C, Kribs M, Abdelfatah A, Chalopin JM. C-reactive protein and cardiovascular disease in peritoneal dialysis patients. *Kidney Int.* (2002) 62:1417–22. doi: 10.1111/j.1523-1755.2002.kid562.x
4. Cobo G, Lindholm B, Stenvinkel P. Chronic inflammation in end-stage renal disease and dialysis. *Nephrol Dial Transplant.* (2018) 33(suppl_3):iii35–40. doi: 10.1093/ndt/gfy175
5. Crépin T, Legendre M, Carron C, Vachey C, Courivaud C, Rebibou JM, et al. Uraemia- induced immune senescence and clinical outcomes in chronic kidney disease patients. *Nephrol Dial Transplant.* (2020) 35:624–32. doi: 10.1093/ndt/gfy276
6. Yoon JW, Gollapudi S, Pahl MV, Vaziri ND. Naive and central memory T-cell lymphopenia in end-stage renal disease. *Kidney Int.* (2006) 70:371–6. doi: 10.1038/sj.ki.5001550

7. Litjens NH, Huisman M, van den Dorpel M, Betjes MG. Impaired immune responses and antigen-specific memory CD4+ T cells in hemodialysis patients. *J Am Soc Nephrol.* (2008) 19:1483–90. doi: 10.1681/ASN.2007090971
8. Crespo J, Sun H, Welling TH, Tian Z, Zou W. T cell anergy, exhaustion, senescence, and stemness in the tumor microenvironment. *Curr Opin Immunol.* (2013) 25:214–21. doi: 10.1016/j.coi.2012.12.003
9. Miller JFAP. The function of the thymus and its impact on modern medicine. *Science.* (2020) 369:eaba2429. doi: 10.1126/science.aba2429
10. Aspinall R, Andrew D. Thymic involution in aging. *J Clin Immunol.* (2000) 20:250–6. doi: 10.1023/A:1006611518223
11. De Haan G, Lazare SS. Aging of hematopoietic stem cells. *Blood.* (2018) 131:479–87. doi: 10.1182/blood-2017-06-746412
12. Wahlestedt M, Erlandsson E, Kristiansen T, Lu R, Brakebusch C, Weissman IL, et al. Clonal reversal of ageing-associated stem cell lineage bias via a pluripotent intermediate. *Nat Commun.* (2017) 8:14533. doi: 10.1038/ncomms14533
13. Beerman I, Maloney WJ, Weissmann IL, Rossi DJ. Stem cells and the aging hematopoietic system. *Curr Opin Immunol.* (2010) 22:500–6. doi: 10.1016/j.coi.2010.06.007
14. Young K, Borikar S, Bell R, Ku?er L, Philip V, Trowbridge JJ. Progressive alterations in multipotent hematopoietic progenitors underlie lymphoid cell loss in aging. *J Exp Med.* (2016) 213:2259–67. doi: 10.1084/jem.20160168
15. Sudo K, Ema H, Morita Y, Nakauchi H. Age-related characteristics of murine hematopoietic stemm cells. *J Exp Med.* (2000) 192:1273–80. doi: 10.1084/jem.192.9.1273
16. Latchney SE, Calvi LM. The aging hematopoietic stem cell niche: phenotypic and functional changes and mechanisms that contribute to hematopoietic aging. *Semin Hematol.* (2017) 54:25–32. doi: 10.1053/j.seminhematol.2016.10.001
17. Sun L, Brown R, Chen S, Zhuge Q, Su DM. Aging induced decline in T-lymphopoiesis is primarly dependent on status of progenitoc niches in the bone marrow and thymus. *Aging.* (2012) 4:606–19. doi: 10.18632/aging.100487
18. Anderson G, Jenkinson EJ. Lymphostromal interactions in thymic development and function. *Nat Rev Immunol.* (2001) 1:31–40. doi: 10.1038/35095500
19. Vaidya HJ, Briones Leon A, Blackburn CC. FOXN1 in thymus organogenesis and development. *Eur J Immunol.* (2016) 46:1826–37. doi: 10.1002/eji.201545814
20. Pantelouris EM. Absence of thymus in a mouse mutant. *Nature.* (1968) 217:370–1. doi: 10.1038/217370a0
21. Rode I, Martins VC, Kublbeck G, Maltry N, Tessmer C, Rodewald HR. Foxn1 protein expression in the developing, aging, and regenerating thymus. *J Immunol.* (2015) 195:5678–87. doi: 10.4049/jimmunol.1502010
22. Sun L, Guo J, Brown R, Amagai T, Zhao Y, Su D-M. Declining expression of a single epithelial autonomous gene accelerates age-related thymic involution. *Aging Cell.* (2010) 9:347–57. doi: 10.1111/j.1474-9726.2010.00559.x
23. Linton PJ, Dorshkind K. Age-related changes in lymphocyte development and function. *Nat Immunol.* (2004) 5:133–9. doi: 10.1038/ni1033
24. Wertheimer AM, Bennett MS, Park B, Uhrlaub JL, Martinez C, Pulko V, et al. Aging and cytomegalovirus infection differentially and jointly affect distinct circulating T cell subsets in humans. *J Immunol.* (2014) 192:2143–55. doi: 10.4049/jimmunol.1301721
25. Hernandez-Segura A, Nehme J, Demaria M. Hallmarks of cellular senescence. *Trends Cell Biol.* (2018) 28:436–53. doi: 10.1016/j.tcb.2018.02.001
26. He S, Sharpless NE. Senescence in health and disease. *Cell.* (2017) 169:1000–11. doi: 10.1016/j.cell.2017.05.015
27. Coppe JP, Patil CK, Rodier F, Sun Y, Munoz DP, Goldstein J, et al. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol.* (2008) 6:2853–68. doi: 10.1371/journal.pbio.0060301
28. Prata L, Ovsyannikova IG, Tchkonia T, Kirkland JL. Senescent cell clearance by the immune system: emerging therapeutic opportunities. *Semin Immunol.* (2018) 40:101275. doi: 10.1016/j.smim.2019.04.003
29. Burton DGA, Stolzing A. Cellular senescence: immunosurveillance and future immunotherapy. *Ageing Res Rev.* (2018) 43:17–25. doi: 10.1016/j.arr.2018.02.001
30. Plowden J, Renshaw-Hoelscher M, Engleman C, Katz J, Sambhara S. Innate immunity in aging: impact on macrophage function. *Aging Cell.* (2004) 3:161–7. doi: 10.1111/j.1474-9728.2004.00102.x
31. Solana R, Tarazona R, Gayoso I, Lesur O, Dupuis G, Fulop T. Innate immunosenescence: effect of aging on cells and receptors of the innate immune system in humans. *Semin Immunol.* (2012) 24:331–41. doi: 10.1016/j.smim.2012.04.008
32. Brunner S, Herndler-Brandstetter D, Weinberger B, Grubeck-Loebenstein B. Persistent viral infections and immune aging. *Ageing Res Rev.* (2011) 10:362–9. doi: 10.1016/j.arr.2010.08.003
33. Coder B, Su DM. Thymic involution beyond T-cell insufficiency. *Oncotarget.* (2015) 6:21777–8. doi: 10.18632/oncotarget.4970
34. Thomas R, Oh J, Wang W, Su D-M. Thymic atrophy creates holes in Treg-mediated immuno-regulation via impairment of an antigen-specific clone. *Immunology.* (2021) 163:478–92. doi: 10.1111/imm.13333
35. Kooman JP, Kotanko P, Schols AM, Shiels PG, Stenvinkel P. Chronic kidney disease and premature ageing. *Nat Rev Nephrol.* (2014) 10:732–42. doi: 10.1038/nrneph.2014.185
36. Ebert T, Pawelzik S-C, Witasp A, Arefin S, Hobson S, Kublickiene K, et al. Inflammation and premature ageing in chronic kidney disease. *Toxins.* (2020) 4:227. doi: 10.3390/toxins12040227
37. George RP, Mehta AK, Perez SD, Winterberg P, Cheeseman J, Johnson B, et al. Premature T cell senescence in pediatric CKD. *J Am Soc Nephrol.* (2017) 28:359–67. doi: 10.1681/ASN.2016010053
38. Levine S, Saltzman A. Are urea and creatinine uremic toxins in the rat? *Ren Fail.* (2001) 23:53–9. doi: 10.1081/JDI-100001283
39. Raskova J, Czerwinski DK, Shea SM et al. Cellular immunity and lymphocyte populations in developing uremia in the rat. *J Exp Pathol.* (1986) 2:229–45.
40. Xiang F, Cao X, Chen X, Zhang Z, Ding X, Zou J, et al. Decreased peripheral naïve T cell number and its role in predicting cardiovascular and infection events in hemodialysis patients. *Front Immunol.* (2021) 12:644627. doi: 10.3389/fimmu.2021.644627
41. Cohen G. Immune dysfunction in uremia 2020. *Toxins.* (2020) 12:439. doi: 10.3390/toxins12070439
42. Girndt M, Trojanowicz B, Ulrich C. Monocytes in uremia. *Toxins.* (2020) 12:340. doi: 10.3390/toxins12050340
43. Jurk D, Wilson C, Passos JF, Oakley F, Correia-Melo C, Greaves L, et al. Chronic inflammation induces telomere dysfunction and accelerates ageing in mice. *Nat Commun.* (2014) 2:4172. doi: 10.1038/ncomms5172
44. Duggal NA, Niemiro G, Harridge SDR, Simpson RJ, Lord JM. Can physical activity ameliorate immunosenescence and thereby reduce age-related multi morbidity? *Nat Rev.* (2019) 19:563–72. doi: 10.1038/s41577-019-0177-9
45. Lee J-Y, Paik Y, Kin JY. Voluntary exercise reverses immune aging induced by oxidative stress in aging mice. *Exp Gerontol.* (2019) 115:148–54. doi: 10.1016/j.exger.2018.08.009
46. Duggal NA, Pollock RD, Lazarus NR, Harridge S, Lord JM. Major features of immunesenescence, including reduced thymic output, are ameliorated by high levels of physical activity in adulthood. *Aging Cell.* (2018) 17:e12750. doi: 10.1111/acel.12750
47. Woods JA, Keylock KT, Lowder T, Vieira VJ, Zelkovich W, Dumich S, et al. Cardiovascular exercise training extends influenza vaccine seroprotection in sedentary older adults: the immune function intervention trial. *J Am Geriatr Soc.* (2009) 57:2183–91. doi: 10.1111/j.1532-5415.2009.02563.x
48. Kohut ML, Arntson BA, Lee W, Rozeboom K, Yoon K-J, Cunnick JE, et al. Moderate exercise improves antibody response to influenza immunization in older adults. *Vaccine.* (2004) 22:2298–306. doi: 10.1016/j.vaccine.2003.11.023
49. Haugen F, Norheim F, Lian H, Wensass AJ, Dueland S, Berg O, et al. IL-7 is expressed and secreted by human skeletal muscle cells. *Am J Physiol Cell Physiol.* (2010) 298:C807–16. doi: 10.1152/ajpcell.00094.2009
50. Rinnow A, Yfanti C, Nielsen S, Akerström TCA, Peijs L, et al. Endurance training enhances skeletal muscle interleukin-15 in human male subjects. *Endocrine.* (2014) 45:271–8. doi: 10.1007/s12020-013-9969-z
51. Whitham, M, Chan MH, Pal M, Matthews VB, Prelovsek O, et al. Contraction- induced interleukin-6 gene transcription in skeletal muscle is

regulated by c- Jun terminal kinase/activator protein-1. *J Biol Chem.* (2012) 287:10771–9. doi: 10.1074/jbc.M111.310581
52. Munoz- Canoves P, Scheele C, Pedersen BK, Serrano AL. Interleukin-6 myokine signaling in skeletal muscle: a double- edged sword? *FEBS J.* (2013) 280:4131–48. doi: 10.1111/febs.12338
53. Starkie R, Ostrowski SR, Jauffred S, Febbraio M, Pedersen BK. Exercise and IL-6 infusion inhibit endotoxin-induced TNF-a production in humans. *FASEB.* (2003) 17:884–6. doi: 10.1096/fj.02-0670fje
54. Ban E, Gagnerault MC, Jammes H, Postel-Vinay MC, Haour F, Dardenne M. Specific binding sites for growth hormone in cultured mouse thymic epithelial cells. *Life Sci.* (1991) 48:2141–8. doi: 10.1016/0024-3205(91)90147-4
55. Geenen V, Achour I, Robert F, Vandersmissen E, Sodoyez JC, Defresne MP, et al. Evidence that insulin-like growth factor 2 (IGF2) is the dominant thymic peptide of the insulin superfamily. *Thymus.* (1993) 21:115–27.
56. Savino W, Postel-Vinay MC, Smaniotto S, Dardenne M. The thymus gland: a target organ for growth hormone. *Scand J Immunol.* (2002) 55:442–52. doi: 10.1046/j.1365-3083.2002.01077.x
57. Hirokawa K, Utsuyama M, Kikuchi Y. Trade off situation between thymus and growth hormone: age-related decline of growth hormone is a cause of thymic involution but favorable for elongation of lifespan. *Biogerontology.* (2016) 17:55–9. doi: 10.1007/s10522-015-9590-z
58. Chen BJ, Cui X, Sempowski GD, Chao NJ. Growth hormone accelerates immune recovery following allogeneic T-cell-depleted bone marrow transplantation in mice. *Exp Hematol.* (2003) 31:953–8. doi: 10.1016/S0301-472X(03)00196-6
59. Hansen BH, Kolte L, Haugaard SB, Dirksen C, Jensen FK, Ryder LP, et al. Improved thymic index, density and output in HIV-infected patients following low-dose growth hormone therapy: a placebo controlled study. *AIDS.* (2009) 23:2123–31. doi: 10.1097/QAD.0b013e3283303307
60. Morrhaye G, Kermani H, Legros JJ, Baron F, Beguin Y, Moutschen M, et al. Impact of growth hormone (GH) deficiency and GH replacement upon thymus function in adult patients. *PLoS ONE.* (2009) 22:e5668. doi: 10.1371/journal.pone.0005668
61. Garcia-Mayor RV, Perez AJ, Gandara A et al. Metabolic clearance rate of biosynthetic growth hormone after endogenous growth hormone suppression with a somatostatin analogue in chronic renal failure patients and control subjects. *Clin Endocrinol.* (1993) 39:337–43. doi: 10.1111/j.1365-2265.1993.tb02374.x
62. Mahesh S, Kaskel F. Growth hormone axis in chronic kidney disease. *Pediatr Nephrol.* (2008) 23:41–8. doi: 10.1007/s00467-007-0527-x
63. Rabkin R, Sun DF, Chen Y, Schaefer F. Growth hormone resistance in uremia, a role for impaired JAK/STAT signaling. *Pediatr Nephrol.* (2005) 20:313–8. doi: 10.1007/s00467-004-1713-8
64. Kamenicky P, Mazziotti G, Lombes M, Giustina A, Chanson P. Growth hormone, insulin-like growth factor-1, and the kidney: pathophysiological and clinical implications. *Endocr Rev.* (2014) 35:234–81. doi: 10.1210/er.2013-1071
65. Frystyk J, Djurhuus CB, Johansen T, Lange M, Smidt K, Christiansen JS. Measurement of free GH and bioactive IGF-1 in non-diabetic haemodialysis patients treated with GH for 7 days. *Nephrol Dial Transplant.* (2012) 27:4211–8. doi: 10.1093/ndt/gfs364
66. Kopple JD, Cheung AK, Christiansen JS, Djurhuus CB, El Nahas M, Feldt-Rasmussen B, et al. Opportunity: a large-scale randomized clinical trial of growth hormone in hemodialysis patients. *Nephrol Dial Transplant.* (2011) 26:4095–103. doi: 10.1093/ndt/gfr363
67. Heng TS, Goldberg GL, Gray DH, Sutherland JS, Chidgey AP, Boyd RL. Effects of castration on thymocyte development in two different models of thymic involution. *J Immunol.* (2005) 175:2982–93. doi: 10.4049/jimmunol.175.5.2982
68. Sutherland JS, Goldberg GL, Hammett MV, Uldrich AP, Berzins SP, Heng TS, et al. Activation of thymic regeneration in mice and humans following androgen blockade. *J Immunol.* (2005) 175:2741–53. doi: 10.4049/jimmunol.175.4.2741
69. Velardi E, Tsai JJ, Holland AM, Wertheimer T, Yu VW, Zakrzewski JL, et al. Sex steroid blockade enhances thymopoiesis by modulating Notch signaling. *J Exp Med.* (2014) 211:2341–9. doi: 10.1084/jem.20131289
70. Rao LV, Cleveland RP, Kimmel RJ, Ataya KM. Gonadotropin-releasing hormone agonist influences absolute levels of lymphocyte subsets *in vivo* in male mice. *Immunol Cell Biol.* (1996) 74:134–43. doi: 10.1038/icb.1996.18
71. Goldberg GL, King CG, Nejat RA, Suh DY, Smith OM, Bretz JC, et al. Luteinizing hormone-releasing hormone enhances T cell recovery following allogeneic bone marrow transplantation. *J Immunol.* (2009) 182:5846–54. doi: 10.4049/jimmunol.0801458
72. Scalea JR, Torabi R, Tena A, Tasaki M, Gillon BC, Moran S, et al. The rejuvenating effects of leuprolide acetate on the aged baboon's thymus. *Transpl Immunol.* (2014) 31:134–9. doi: 10.1016/j.trim.2014.09.001
73. Min H, Montecino-Rodriguez E, Dorshkind K. Reassessing the role of growth hormone and sex steroids in thymic involution. *Clin Immunol.* (2006) 118:117–23. doi: 10.1016/j.clim.2005.08.015
74. Griffith AV, Fallahi M, Venables T, Petrie HT. Persistent degenerative changes in thymic organ function revealed by an inducible model of organ regrowth. *Aging Cell.* (2012) 11:169–77. doi: 10.1111/j.1474-9726.2011.00773.x
75. Page ST, Plymate SR, Bremner WJ, Matsumoto AM, Hess DL, Lin DW, et al. Effect of medical castration on CD4+ CD25+ T cells, CD8+ T cell IFN- gamma expression, and NK cells: a physiological role for testosterone and/ or its metabolites. *Am J Physiol Endocrinol Metab.* (2006) 290:E856–63. doi: 10.1152/ajpendo.00484.2005
76. Dragin N, Bismuth J, Cizeron-Clairac G, Biferi MG, Berthault C, Serraf A, et al. Estrogen-mediated downregulation of AIRE influences sexual dimorphism in autoimmune diseases. *J Clin Invest.* (2016) 126:1525–37. doi: 10.1172/JCI81894
77. Chaudhry MS, Velardi E, Dudakov JA, van den Brick MRM. Thymus: the next(re)generation. *Immunol Rev.* (2016) 271:56–71. doi: 10.1111/imr.12418
78. Puel A, Ziegler SF, Buckley RH, Leonard WJ. Defective IL7R expression in T(-)B(-)NK(+) severe combined immunodeficiency. *Nat Genet.* (1998) 20:394–7. doi: 10.1038/3877
79. Schluns KS, Kieper WC, Jameson SC, Lefrançois L. Interleukin-7 mediates the homeostasis of naïve and memory CD8 T cells *in vivo*. *Nat Immunol.* (2000) 1:426–32. doi: 10.1038/80868
80. Lundström W, Fewkes N, Mackall CL. IL-7 in human health and disease. *Semin Immunol.* (2012) 24:218–24. doi: 10.1016/j.smim.2012.02.005
81. de Roquetaille C, Monneret G, Gossez M, Venet F. IL-7 and its beneficial role in sepsis-induced T lymphocyte dysfunction. *Crit Rev Immunol.* (2018) 38:433–51. doi: 10.1615/CritRevImmunol.2018027460
82. Thiebaut R, Jarne A, Routy JP, Sereti I, Fischl M, Ive P, et al. Repeated cycles of recombinant human interleukin 7 in HIV-infected patients with low CD4 T-cell reconstitution on antiretroviral therapy: results of 2 phase II multicenter studies. *Clin Infect Dis.* (2016) 62:1178–85. doi: 10.1093/cid/ciw065
83. Sheikh V, Porter BO, DerSimonian R, Kovacs SB, Thompson WL, Perez-Diez A, et al. Administration of interleukin-7 increases CD4 T cells in idiopathic CD4 lymphocytopenia. *Blood.* (2016) 127:977–88. doi: 10.1182/blood-2015-05-645077
84. Sportes C, Hakim FT, Memon SA, Zhang H, Chua KS, Brown MR, et al. Administration of rhIL-7 in humans increases *in vivo* TCR repertoire diversity by preferential expansion of naive T cell subsets. *J Exp Med.* (2008) 205:1701–14.
85. Levy Y, Lacabaratz C, Weiss L, Viard JP, Goujard C, Lelievre JD, et al. Enhanced T cell recovery in HIV1-infected adults through IL-7 treatment. *J Clin Invest.* (2009) 119:997–1007. doi: 10.1172/JCI38052
86. Pahl MV, Gollapudi S, Sepassi L, Gollapudi P, Elahimehr R, Vaziri ND. Effect of end-stage renal disease on B-lymphocyte subpopulations, IL-7, BAFF, and BAFF-receptor expression. *Nephrol Dial Transplant.* (2010) 25:205–12. doi: 10.1093/ndt/gfp397
87. Duhen T, Geiger R, Jarrossay D, Lanzavecchia A, Sallusto F. Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells. *Nat Immunol.* (2009) 10:857–63. doi: 10.1038/ni.1767
88. Wolk K, Kunz S, Witte E, Friedrich M, Asadullah K, Sabat R. IL-22 increases the innate immunity of tissues. *Immunity.* (2004) 21:241–54. doi: 10.1016/j.immuni.2004.07.007
89. Dudakov JA, Hanash AM, Jenq RR, Young LF, Ghosh A, Singer NV, et al. Interleukin-22 drives endogenous thymic regeneration in mice. *Science.* (2012) 336:91–5. doi: 10.1126/science.1218004

90. Pan B, Zhang F, Lu Z, Li L, Shang L, Xia F, et al. Donor T-cell-derived interleukin-22 promotes thymus regeneration and alleviates chronic graft-versus-host disease in murine allogeneic hematopoietic cell transplant. *Int Immunopharmacol.* (2019) 67:194–201. doi: 10.1016/j.intimp.2018.12.023
91. Pan B, Wang D, Li L, Shang L, Xia F, Zhang F, et al. IL-22 accelerates thymus regeneration via stat3/Mcl-1 and decreases chronic graft-versus-host disease in mice after allotransplants. *Biol Blood Marrow Transplant.* (2019) 25:1911–9. doi: 10.1016/j.bbmt.2019.06.002
92. Shang L, Duah M, Xu Y, Liang Y, Wang D, Xia F, et al. Dynamic plasma Il-22 is an indicator of thymic output after allogeneic hematopoietic cell transplantation. *Life Sci.* (2021) 265:118849. doi: 10.1016/j.lfs.2020.118849
93. Alpdogan O, Hubbard VM, Smith OM, Patel N, Lu S, Goldberg GL, et al. Keratinocyte growth factor (KGF) is required for postnatal thymic regeneration. *Blood.* (2006) 107:2453–60. doi: 10.1182/blood-2005-07-2831
94. Min D, Panoskaltsis-Mortari A, Kuro-O M, Holländer GA, Blazar BR, Weinberg KI. Sustained thymopoiesis and improvement in functional immunity induced by exogenous KGF administration in murine models of aging. *Blood.* (2007) 109:2529–37. doi: 10.1182/blood-2006-08-043794
95. Seggewiss R, Lore K, Guenaga FJ, Pittaluga S, Mattapallil J, Chow CK, et al. Keratinocyte growth factor augments in dose-dependent manner immune reconstitution after autologous hematopoietic progenitor cell transplantation in rhesus macaques. *Blood.* (2007) 110:441–9. doi: 10.1182/blood-2006-12-065623
96. Wils E-J, Aerts-Kaya FSF, Rombouts EJC, van Mourik I, Rijken-Schelen A, Visser TP, et al. Keratinocyte growth factor and stem cell factor to improve thymopoiesis after autologous CD34+ cell transplantation in rhesus macaques. *Biol Blood Marrow Transplant.* (2012) 18:55–65. doi: 10.1016/j.bbmt.2011.09.010
97. Coles AJ, Azzopardi L, Kousin-Ezewu O, Kaur Mullay H, Thompson SAJ, Jarvis L, et al. Keratinocyte growth factor impairs human thymic recovery from lymphopenia. *JCI Insight.* (2019) 5:e125377. doi: 10.1172/jci.insight.125377
98. Jacobson JM, Wang H, Bordi R, Zheng L, Gross BH, Landay AL, et al. A randomized controlled trial of palifermin (recombinant human keratinocyte growth factor) for the treatment of inadequate CD4+ T-lymphocyte recovery in patients with HIV-1 infection on antiretroviral therapy. *J Acquir Immune Defic Syndr.* (2014) 66:399–406. doi: 10.1097/QAI.0000000000000195
99. McAuley DF, Cross LjM, Hamid U, Gardner E, Elborn JS, Cullen KM et al. Keratinocyte growth factor for the treatment of the acute respiratory distress syndrome (KARE): a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet Respir Med.* (2017) 5:484–91. doi: 10.1016/S2213-2600(17)30171-6
100. Kim MJ, Miller CM, Shadrach JL, Wagers AJ, Serwold T. Young, proliferative thymic epithelial cells engraft and function in aging thymuses. *J Immunol.* (2015) 194:4784–95. doi: 10.4049/jimmunol.1403158
101. Bredenkamp N, Ulyanchenko S, O'Neill KE, Manley NR, Vaidya HJ, Blackburn CC. An organized and functional thymus generated from FOXN1-reprogrammed fibroblasts. *Nat Cell Biol.* (2014) 16:902–8. doi: 10.1038/ncb3023
102. Oh J, Wang W, Thomas R, Su D-M. Thymic rejuvenation via FOXN1-reprogrammed embryonic fibroblasts (FREFs) to counteract age-related inflammation. *JCI Insight.* (2020) 5:e140313. doi: 10.1172/jci.insight.140313
103. Bredenkamp N, Nowell CS, Blackburn CC. Regeneration of the aged thymus by a single transcription factor. *Development.* (2014) 141:1627–37. doi: 10.1242/dev.103614
104. Song Y, Su M, Zhu J, Di W, Liu Y, Hu R, et al. FOXN1 recombinant preotein enhances T-cell regeneration after hematopoietic stem cell transplantation in mice. *Eur J Immunol.* (2016) 46:1518–28. doi: 10.1002/eji.201546196
105. Honda K, Littman DR. The microbiome in infectious disease and inflammation. *Ann Rev Immunol.* (2012) 30:759–95. doi: 10.1146/annurev-immunol-020711-074937
106. de la Cuesta-Zuluaga J, Kelley ST, Chen Y, Escobar JS, Mueller NT, Ley RE, et al. Age- and sex-dependent patterns of gut microbial diversity in human adults. *Msystems.* (2019) 4:e00261–19. doi: 10.1128/mSystems.00261-19
107. Ghosh TS, Das M, Jeffery IB, O'Toole PW. Adjusting for age improves identification of gut microbiome alterations in multiple diseases. *Elife.* (2020) 9:e50240. doi: 10.7554/eLife.50240
108. Thevaranjan N, Puchta A, Schultz C, Naidoo A, Szamosi JC, Verschoor CP, et al. Age-associated microbial dysbiosis promotes intestinal permeability, systemic inflammation, and macrophage dysfunction. *Cell Host Microbe.* (2017) 12:455–66. doi: 10.1016/j.chom.2017.03.002
109. Mabbott NA. A breakdown in communication? Understanding the effects of aging on the human small intestine epithelium. *Clin Sci.* (2015) 129:529–31. doi: 10.1042/CS20150364
110. Haghikia A, Jörg S, Duscha A, Berg J, Manzel A, Waschbisch A, et al. Dietary fatty acids directly impact central nervous system autoimmunity via the small intestine. *Immunity.* (2015) 43:817–29. doi: 10.1016/j.immuni.2015.09.007
111. Rosser EC, Piper CJM, Matei DE, Blair PA, Rendeiro AF, Orford M, et al. Microbiota-derived metabolites suppress arthritis by amplifying aryl-hydrocarbon receptor activation in regulatory B cells. *Cell Metab.* (2020) 31:837–51. doi: 10.1016/j.cmet.2020.03.003
112. Mane J, Pedrosa E, Loren V, Gassull MA, Espadaler J, Cune J, et al. A mixture of lactobacillus plantarum CECT 7315 and CECT 7316 enhances systemic immunity in elderly subjects. A dose-response, double-blind, placebo-controlled, randomized pilot trial. *Nutr Hosp.* (2011) 26:228–35. doi: 10.1016/j.cmet-2020-03-003
113. Finamore A, Roselli M, Donini L, Brasili E, Rami R, Carnevali P, et al. Supplementation with bifidobacterium longum Bar33 and lactobacillus helveticus Bar13 mixture improves immunity in elderly humans (over 75 years) and aged mice. *Nutrition.* (2019) 63–4:184–92. doi: 10.1016/j.nut.2019.02.005
114. Akatsu H, Nagafuchi S, Kurihara R, Okuda K, Kanesaka T, Ogawa N, et al. Enhanced vaccination effect against influenza by prebiotics in elderly patients receiving enteral nutrition. *Geriatr Gerontol Int.* (2016) 16:205–13. doi: 10.1111/ggi.12454
115. Boge T, Remigy M, Vaudaine S, Tanguy J, Bourdet-Sicard R, van der Werf S. A probiotic fermented dairy drink improves antibody response to influenza vaccination in the elderly in two randomised controlled trials. *Vaccine.* (2009) 27:5677–84. doi: 10.1016/j.vaccine.2009.06.094
116. Lei WT, Shih PC, Liu SJ, Lin CY, Yeh TL. Effects of probiotics and prebiotics on immune response to influenza vaccination in adults: a systematic review and meta-analysis of randomized controlled trials. *Nutrients.* (2017) 9:9. doi: 10.3390/nu9111175
117. Wang B, Hylwka T, Smieja M, Surrette M, Bowdish DME, Loeb M. Probiotics to prevent respiratory infections in nursing homes: a pilot randomized controlled trial. *J Am Geriatr Soc.* (2018) 66:1346–52. doi: 10.1111/jgs.15396
118. Zhao J, Ning X, Liu B, Dong R, Bai M, Sun S. Specific alterations in gut microbiota in patients with chronic kidney disease: an updated systematic review. *Ren Fail.* (2021) 43:102–12. doi: 10.1080/0886022X.2020.1864404
119. Felizardo RJ, Castoldi A, Andrade-Oliveira V, Câmera NO. The microbiota and chronic kidney disease: a double-edged sword. *Clin Transl Immunol.* (2016) 5:e86. doi: 10.1038/cti.2016.36
120. Lau WL, Savoj J, Nakata MB, Vaziri ND. Altered microbiome in chronic kidney disease: systemic effects of gut-derived uremic toxins. *Clin Sci.* (2018) 132:509–22. doi: 10.1042/CS20171107
121. Vaziri ND, Wong J, Pahl M, Piceno YM, Yuan J, DeSantis TZ, et al. Chronic kidney disease alters intestinal microbial flora. *Kidney Int.* (2013) 83:308–15. doi: 10.1038/ki.2012.345
122. Carron C, Pais de Barros JP, Gaiffe E, Deckert V, Adda-Rezig H, Roubiou C, et al. End-stage renal disease-associated gut baterial translocation: evolution and impact on chronic inflammation and acute rejection after renal transplantation. *Front Immunol.* (2019) 10:1630. doi: 10.3389/fimmu.2019.01630
123. Adda-Rezig H, Carron C, Pais de Barros JP, Choubley H, Charron E, Rérole A-L, et al. New insights on end-stage renal disease and healthy individual gut bacterial translocation: different carbon composition of lipopolysaccharides and different impact on monocyte inflammatory response. *Front Immunol.* (2021) 10. doi: 10.3389/fimmu.2021.658404
124. Glorieux G, Gryp T, Perna A. Gut-derived metabolites and their role in immune dysfunction in chronic kidney disease. *Toxins.* (2020) 12:245. doi: 10.3390/toxins12040245
125. Chiu YL Shu KH, Yang FJ, Chou TY, Chen PM, Lay FY, et al. A comprehensive characterization of aggravated aging-related changes in T lymphocytes and monocytes in end-stage renal disease. The iESRD study. *Immun Ageing.* (2018) 15:27. doi: 10.1186/s12979-018-0131-x

126. Di Lorio BR, Rochetti MT, De Angelis M, Cosola C, Marzoco S, Di Micco L, et al. Nutritional therapy modulates intestinal microbiota and reduces serum levls of total and free indoxyl sulfate and p-cresyl sulfate in chronic kidney disease (Medika study). *J Clin Med.* (2019) 8:1424. doi: 10.3390/jcm8091424
127. Crepin T, Carron C, Roubiou C, Gaugle B, Gaiffe E, Simula-Faivre D, et al. ATG-induced accelerated immune senescence: clinical implications in renal transplant recipients. *Am J Transplant.* (2015) 15:1028–38. doi: 10.1111/ajt.13092
128. Meijers RWJ, Litjens NHR, de Wit EA, Langerak AW, Baan CC, Betjes MGH. Uremia-associated immunological aging is stably imprinted in the T-cell system and not reversed by kidney transplantation. *Transplant Int.* (2014) 27:1272–84. doi: 10.1111/tri.12416
129. Kaebisch EM, Cho M-Y, Oh Y-S, Olfe Ll, Szyska M, Becker SC, et al. Cytotoxic effects of rabbit anti-thymocyte globulin preparations on primary human thymic epithelial cells. *Transplantation.* (2019) 103:2234–44. doi: 10.1097/TP.0000000000002799
130. Preville X, Flacher M, LeMauff B, Beauchard S, Davelu P, Tiollier J, et al. Mechanisms involved in antithymocyte globulin immunosuppressive activity in a nohuman primate model. *Transplantation.* (2001) 71:460–8. doi: 10.1097/00007890-200102150-00021
131. Pan H, Gazarian A, Mollet I, Mathias V, Dubois V, Sobh M, et al. Lymphodepletive effects of rabbit anti-pig thymocyte globulin in neonatal swines. *Transplant Immunol.* (2016) 39:74–83. doi: 10.1016/j.trim.2016.08.005
132. Ducloux D, Bamoulid J, Crepin T, Rebibou J-M, Courivaud C, Saas P. Posttransplant immune activation: innocent bystander or insidious culprit of posttransplant accelerated atherosclerosis. *Cell Transplantation.* (2017) 26:1601–9. doi: 10.1177/0963689717735404
133. Lambie M, Chess J, Donovan L Kim YL, Do JY, Lee HB, et al. Independent effects of systemic and peritoneal inflammation on peritoneal dialysis survival. *J Am Soc Nephrol.* (2013) 24:2071–80. doi: 10.1681/ASN.2013030314
134. Pecoits-Filho R, Carvalho MJ, Stenvinkel P, Lindholm B, Heimbürger O. Systemic and intraperitoneal interleukin- 6 system during the first year of peritoneal dialysis. *Perit Dial Int.* (2006) 26:53–63. doi: 10.1177/089686080602600109
135. Cho JH, Hur IK, Kim CD, Park SH, Ryu HM, Yook JM, et al. Impact of systemic and local peritoneal inflammation on peritoneal solute transport rate in new peritoneal dialysis patients: a year prospective study. *Nephrol Dial Transplant.* (2010) 25:1964–73. doi: 10.1093/ndt/gfp767
136. Oldani S, Finazzi S, Botazzi B, Garlanda C, Baldassarre E, Valaperta S, et al. Plasma pentraxin-3 as a marker of biocompatibility in hemodialysis patients. *J Nephrol.* (2012) 25:120–6. doi: 10.5301/JN.2011.8432
137. Yamamoto T, Nascimento MM, Hayashi SY, Qureshi AR, Waniewski J, Brodin LA, et al. Changes in circulating biomarkers during a single hemodialysis session. *Hemodialysis Int.* (2013) 17:59–66. doi: 10.1111/j.1542-4758.2012.00720.x
138. Bitla AR, Reddy PE, Manohar SM, Vishnubhotla SV, Pemmaraju Venkata Lakshmi Narasimha SR. Effect of a single hemodialysis session on inflammatory markers. *Hemodialysis Int.* (2010) 14:411–7. doi: 10.1111/j.1542-4758.2010.00491.x
139. Gollapudi P, Yoon JW, Gollapudi S, Pahl, MV, Vaziri ND. Leukocyte toll-like receptor expression in end-stage kidney disease. *Am J Nephrol.* (2010) 31:247–54. doi: 10.1159/000276764
140. Ando M, Shibuya A, Tsuchiya K, Akiba T, Nitta K. Reduced expression of Toll-like receptor 4 contributes to impaired cytokine response of monocytes in uremic patients. *Kidney Int.* (2006) 70:358–62. doi: 10.1038/sj.ki.5001548
141. Ducloux D, Legendre M, Bamoulid J, Rebibou JM, Saas P, Courivaud C, et al. ESRD-associated immune phenotype depends on dialysis modality and iron status: clinical implications. *Immun Ageing.* (2018) 15:16. doi: 10.1186/s12979-018-0121-z
142. Xu D, Erickson S, Szeps M, Gruber A, Sangfelt O, Einhorn S, et al. Interferon alpha down-regulates telomerase reverse transcriptase and telomerase activity in human malignant and nonmalignant hematopoietic cells. *Blood.* (2000) 96:4313–8. doi: 10.1182/blood.V96.13.4313.h8004313_4313_4318
143. Reed JR, Vukmanovic-Stejic M, Fletcher JM, Soares MV, Cook JE, Orteu CH, et al. 2004. Telomere erosion in memory T cells induced by telomerase inhibition at the site of antigenic challenge *in vivo*. *J Exp Med.* (2004) 199:1433–43. doi: 10.1084/jem.20040178
144. Dell'Oglio MP, Simone S, Ciccone M, Corciulo R, Gesualdo M, Zito A, et al. Neutrophil-dependent pentraxin-3 and reactive oxygen species production modulate endothelial dysfunction in haemodialysis patients. *Nephrol Dial Transplant.* (2017) 32:1540–9. doi: 10.1093/ndt/gfw363
145. Efthymios P., Spiridoula M, Periklis K, Andreas C, Sotiris T, Theodoros E, et al. The effect of dialysis modality and membrane performance on native immunity in dialysis patients. *Prilozi.* (2019) 40:25–32. doi: 10.2478/prilozi-2019-0011
146. Chonchol, M. Neutrophil dysfunction and infection risk in end-stage renal disease. *Semin Dial.* (2006) 19:291–6. doi: 10.1111/j.1525-139X.2006.00175.x
147. Poppelaars F, da Costa MG, Berger S., Assa S, Meter-Arkema AH, Daha MR, et al. Strong predictive value of mannose-binding lectin levels for cardiovascular risk of hemodialysis patients. *J Transl Med.* (2016) 14:236. doi: 10.1186/s12967-016-1004-8
148. Lines SW, Richardson VR, Thomas B, Dunn EJ, Wright MJ, Carter AM. Complement and cardiovascular disease—the missing link in haemodialysis patients. *Nephron.* (2016) 132:5–14. doi: 10.1159/000442426
149. Losappio V, Franzin R, Infante B, Godeas G, Gesualdo L, Fersini A, et al. Molecular mechanisms of premature aging in hemodialysis: the complex interplay between innate and adaptive immune dysfunction. *Int J Mol Sci.* (2020) 21:3422. doi: 10.3390/ijms21103422
150. Sepe V, Gregorini M, Rampino T, Esposito P, Coppo R, Galli F, et al. Vitamin e-loaded membrane dialyzers reduce hemodialysis inflammaging. *BMC Nephrol.* (2019) 20:412. doi: 10.1186/s12882-019-1585-6
151. Griffith AV, Venables T, Shi J, Farr A, van Remmen H, Szweda L, et al. Metabolic damage and premature thymus aging caused by stromal catalase deficiency. *Cell Rep.* (2015) 12:1071–9. doi: 10.1016/j.celrep.2015.07.008
152. Reis T, Anwar S, de Assis da Rocha Neves F, Ronco C. Disruptive technologies for hemodialysis: medium and high cutoff membranes. Is the future now. *Braz J Nephrol.* (2021) 12. doi: 10.1590/2175-8239-jbn-2020-0273
153. Zickler D, Schindler R, Willy K, Martus P, Pawlak M, Storr M, et al. Medium cut-off (MCO) membranes reduce inflammation in chronic dialysis patients-a randomized controlled clinical trial. *PLoS ONE.* (2017) 12:e0169024. doi: 10.1371/journal.pone.0169024
154. Aoike I. Clinical significance of protein adsorbable membranes—long-term clinical effects and analysis using a proteomic technique. *Nephrol Dial Transplant.* (2007) 22(Suppl. 5):vl3–9. doi: 10.1093/ndt/gfm295
155. Galli F, Benedetti S, Floridi A, Canestrari F, Piroddi M, Buoncristiani E, et al. Glycoxidation and inflammatory markers in patients on treatment with PMMA-based protein-leaking dialyzers. *Kidney Int.* (2005) 67:750–9. doi: 10.1111/j.1523-1755.2005.67138.x
156. Contin-Bordes C, Lacraz A, de Précigout V. Potential role of the soluble form of CD40 in deficient immunological function of dialysis patients: new findings of its amelioration using polymethylmethacrylate (PMMA) membrane. *NDT Plus.* (2010) 3(Suppl 1):i20–7. doi: 10.1093/ndtplus/sfq033
157. Wetmore JB, Peng Y, Monda KL, Kats AM, Kim DH, Bradbury BD, et al. Trends in anemia management practices in patients receiving hemodialysis and peritoneal dialysis: a retrospective cohort analysis. *Am J Nephrol.* (2015) 41:354–61. doi: 10.1159/000431335
158. Liakopoulos V, Roumeliotis S, Gomy X, Dounousi E, Mertens PR. Oxidative stress in hemodialysis patients: a review of literature. *Oxid Med Cell Longev.* (2017) 2017:3081856. doi: 10.1155/2017/3081856
159. Shin C, Baik I. Transferrin saturation concentrations associated with telomeric ageing: a population-based study. *Br J Nutr.* (2017) 117:1693–701. doi: 10.1017/S0007114517001696
160. Murillo-Ortiz B, Ramirez Emiliano J, Hernandez Vazquez WI, Martinez-Garza S, Solorio-Meza S, Albarran-Tamayo F, et al. Impact of oxidative stress in premature aging and iron overl oad in hemodialysis patients.

Oxid Med Cell Longev. (2016) 2016:1578235. doi: 10.1155/2016/1578235

161. Kepinska M, Szyller J, Milnerowicz H. The influence of oxidative stress induced by iron on telomere length. *Environ Toxicol Pharmacol.* (2015) 40:931–5. doi: 10.1016/j.etap.2015.10.002
162. Xie Y, Hou W, Song X, Yu Y, Huang J, Sun X, et al. Ferroptosis: process and function. *Cell death Diff.* (2016) 23:369–79. doi: 10.1038/cdd.2015.158

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

ORIGINAL RESEARCH
published: 27 September 2021
doi: 10.3389/fmed.2021.705159

Effect of Kidney Transplantation on Accelerated Immunosenescence and Vascular Changes Induced by Chronic Kidney Disease

Noemi Ceprian[1†], Gemma Valera[2†], Jara Caro[3], Claudia Yuste[3], Nadia Serroukh[1], Ignacio González de Pablos[4], Carlos Oliva[5], Andrea Figuer[2], Manuel Praga[3], Matilde Alique[2], Rafael Ramirez[2], Enrique Morales[3†] and Julia Carracedo[1†]*

[1] Departamento de Genética, Fisiología y Microbiología, Universidad Complutense de Madrid, Instituto de Investigaci3n Sanitaria Hospital 12 de Octubre (imas12), Madrid, Spain, [2] Departamento Biología de Sistemas (Unidad Fisiología), Facultad de Medicina, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Alcalá de Henares, Madrid, Spain, [3] Departamento de Nefrología, Hospital Universitario 12 de Octubre, Instituto de Investigación Sanitaria 12 de Octubre, Madrid, Spain, [4] Departamento de Nefrología, Hospital Universitario 12 de Octubre, Madrid, Spain, [5] Departamento de Genética, Fisiología y Microbiología, Universidad Complutense de Madrid, Madrid, Spain

Kidney transplantation is the best option for patients with end-stage renal disease. Despite the improvement in cardiovascular burden (leading cause of mortality among patients with chronic kidney disease), cardiovascular adverse outcomes related to the inflammatory process remain a problem. Thus, the aim of the present study was to characterize the immune profile and microvesicles of patients who underwent transplantation. We investigated the lymphocyte phenotype (CD3, CD4, CD8, CD19, and CD56) and monocyte phenotype (CD14, CD16, CD86, and CD54) in peripheral blood, and endothelium-derived microvesicles (annexin V+CD31+CD41–) in plasma of patients with advanced chronic kidney disease ($n = 40$), patients with transplantation ($n = 40$), and healthy subjects ($n = 18$) recruited from the University Hospital "12 de Octubre" (Madrid, Spain). Patients with kidney transplantation had B-cell lymphopenia, an impairment in co-stimulatory (CD86) and adhesion (CD54) molecules in monocytes, and a reduction in endothelium-derived microvesicles in plasma. The correlations between those parameters explained the modifications in the expression of co-stimulatory and adhesion molecules in monocytes caused by changes in lymphocyte populations, as well as the increase in the levels of endothelial-derived microvesicles in plasma caused by changes in lymphocyte and monocytes populations. Immunosuppressive treatment could directly or indirectly induce those changes. Nevertheless, the particular characteristics of these cells may partly explain the persistence of cardiovascular and renal alterations in patients who underwent transplantation, along with the decrease in arteriosclerotic events compared with advanced chronic kidney disease. In conclusion, the expression of adhesion molecules by monocytes and endothelial-derived microvesicles is related to lymphocyte alterations in patients with kidney transplantation.

Keywords: chronic kidney disease, immunity, immunosenescence, microvesicles, renal transplantation

OPEN ACCESS

Edited by:
Milena Ivanova Ivanova - Shivarova, Aleksandrovska University Hospital, Bulgaria

Reviewed by:
Alexander E. Berezin, Zaporizhia State Medical University, Ukraine
Mohammad Hossein Karimi, Shiraz University of Medical Sciences, Iran

***Correspondence:**
Julia Carracedo
julcar01@ucm.es

[†] These authors have contributed equally to this work

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *04 May 2021*
Accepted: *31 August 2021*
Published: *27 September 2021*

Citation:
Ceprian N, Valera G, Caro J, Yuste C, Serroukh N, González de Pablos I, Oliva C, Figuer A, Praga M, Alique M, Ramirez R, Morales E and Carracedo J (2021) Effect of Kidney Transplantation on Accelerated Immunosenescence and Vascular Changes Induced by Chronic Kidney Disease. Front. Med. 8:705159. doi: 10.3389/fmed.2021.705159

INTRODUCTION

Chronic kidney disease (CKD) is one of the leading causes of mortality and morbidity in developed countries (1). This pathology has a high frequency, affecting ∼9% of the population worldwide (2). The incidence of CKD is expected to increase in the future (3) due to the increase in the prevalence of risk factors, such as hypertension and diabetes mellitus (2, 4, 5).

As CKD progresses and kidney function becomes less effective, various substances collectively termed uremic retention solutes accumulate in the body; those that exert adverse biological effects are termed uremic toxins. Uremic toxins are thought to contribute to inflammation, immune dysfunction, vascular disease, platelet dysfunction and increased bleeding risk, dysbiosis in the gut including increased translocation of bacteria, altered drug metabolism, as well as CKD progression (6–11). In addition, the risk of a cardiovascular event increases with decreasing renal glomerular filtration rate (GFR) and the occurrence of albuminuria (6, 12–14). The inflammatory state in advanced chronic kidney disease (ACKD) due to the inflammatory process in the kidney (15, 16) and the increase in excretion products in blood (e.g., uremic toxins or proinflammatory compounds) is particularly interesting (11, 17, 18). Both events lead to low-grade inflammation, similar to the basal inflammation observed in aging (4, 11, 17), which can be identified as inflamm-aging (19, 20). This low-grade inflammation is also associated with a worsening response to infections (21–23), an increased incidence of cancer (24, 25), and senescent phenotypes in immune cells and the vascular endothelium (4, 17, 18, 26–30). This inflammation and cellular senescence entail the development of associated pathologies, such as cardiovascular disease, which is the primary cause of mortality in CKD (4, 31). Proinflammatory monocytes (intermediate and non-classical) play a crucial role in the development of this pathology (4, 27, 30–33). In recent years, the extracellular vesicles—small particles which serve as a means of communication between cells—have captured the attention of researchers (34–37). The adhesion of monocytes to the vascular endothelium leads to release of proangiogenic factors and extracellular vesicles, including microvesicles (MV), by the endothelial cells, thereby inducing vascular damage (4, 27, 34, 38).

Most patients reaching end-stage kidney disease are treated with either dialysis or kidney transplantation (KT), which is currently the best available therapeutic option (39–41). However, KT does not entirely solve the problem primarily because the leading cause of CKD continues to affect the patient and prolongs the associated pathologies. Furthermore, other conditions, such as nephrotoxicity (42), anemia (43), oxidative stress (44), cardiovascular alterations (45, 46), or mineral-bone alterations (47) persist in patients who underwent transplantation. Moreover, immunosuppression (42), which is fundamental for avoiding transplant rejection, may modulate the low-grade basal inflammation. Currently, this potential relationship has not been extensively studied in situations of normal renal transplantation.

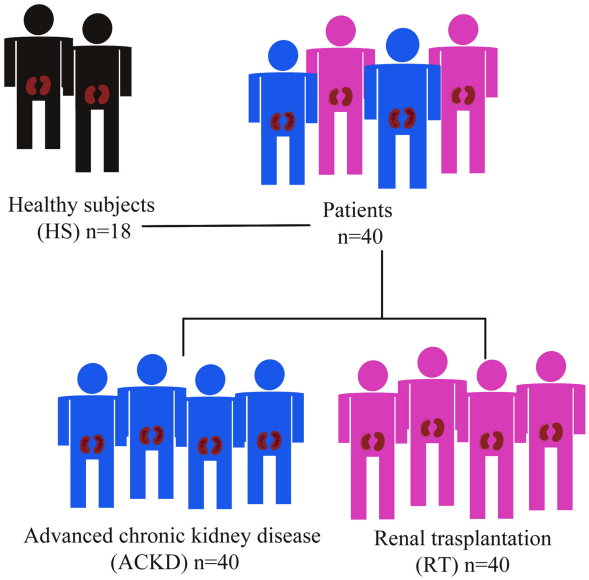


FIGURE 1 | Description of cross-sectional study population.

Numerous alterations associated with ACKD and its different treatments have been identified, including those that affect the immune and vascular systems. However, the approach through which these alterations can be corrected, at least partially by the KT procedure, is not well-established. Thus, the aim of the present study was to characterize the immune profile and MVs of patients with KT. This knowledge can be advantageous in designing strategies for monitoring patients and, above all, assessing the effectiveness of different treatments.

MATERIALS AND METHODS

Study Population

We carried out a cross-sectional analysis involving 80 patients with CKD and 18 healthy subjects (HS) to establish standard criteria (**Figure 1**). Forty patients had stage 4–5 CKD, while the remaining 40 had received initial KT at least 6 months prior to sample collection. Patients with neoplasms, infections, and inflammatory or active autoimmune diseases were excluded. All patients were recruited at the Department of Nephrology, University Hospital "12 de Octubre" (Madrid, Spain). All procedures were performed according to the World Medical Association's Declaration of Helsinki and the protocol was approved by the Instituto de Investigación Sanitaria Hospital 12 de Octubre Ethics Committee (CEI: 17/407).

Serum Sample Collection

Peripheral blood samples were obtained in ethylenediaminetetraacetic acid-coated tubes during routine medical reviews. All samples were analyzed within 18 h

Abbreviations: ACKD, advanced chronic kidney disease; CKD, chronic kidney disease; GFR, glomerular filtration rate; HS, healthy subjects; MV, microvesicles; NK, natural killer.

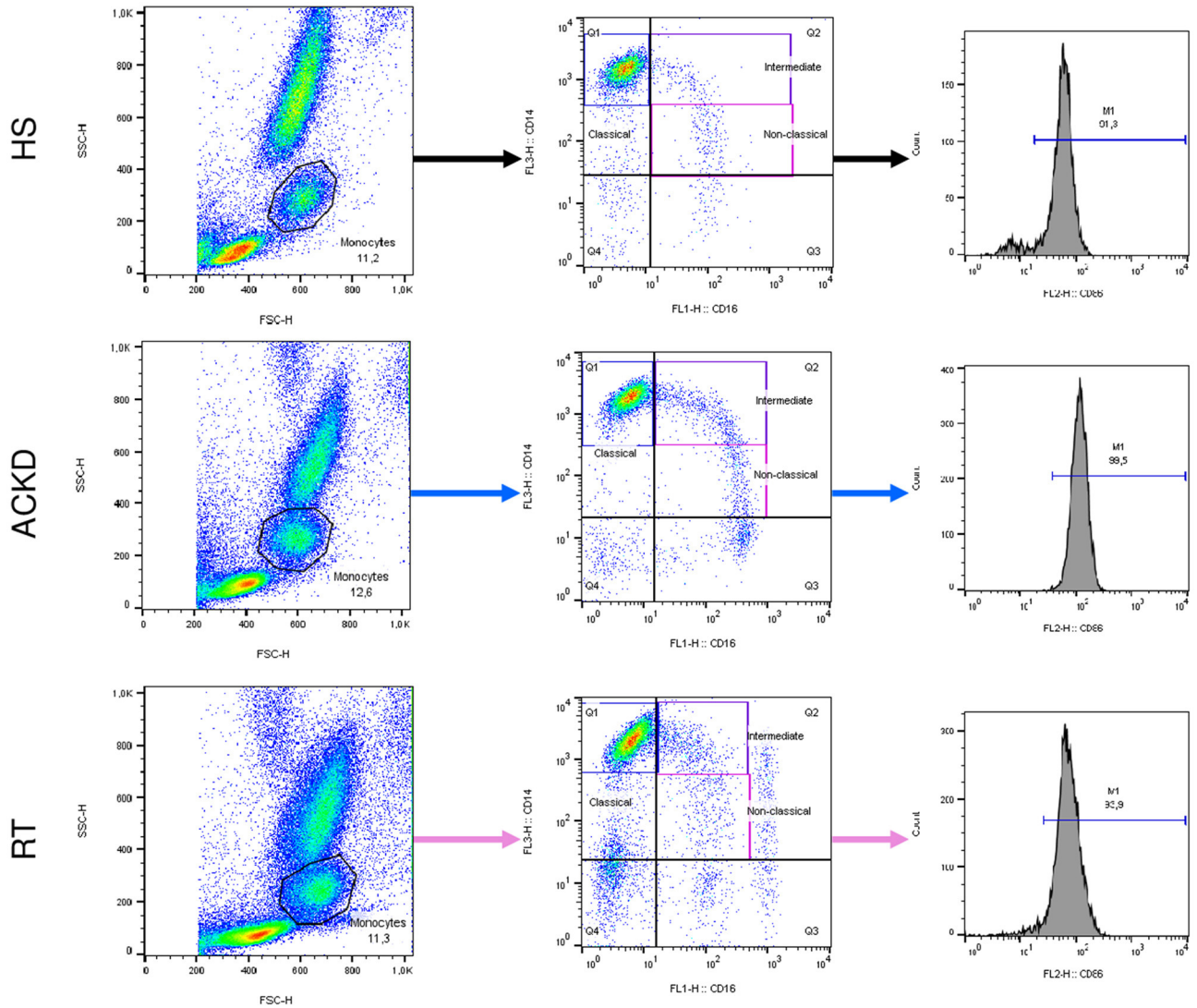


FIGURE 2 | Representative flow cytometry findings of monocyte subsets and CD54/CD86 expression in the three groups: healthy subjects (HS), patients with advanced chronic kidney disease (ACKD), and patients with renal transplantation (RT). Monocyte subpopulations were assessed within the FSC-height/SSC-height. The classical (CD14++CD16–), intermediate (CD14++CD16+), and non-classical (CD14+CD16+) monocytes were evaluated using anti-CD16-FITC and anti-CD14–TRICOLOR. For each subpopulation, the expression of CD54 or CD86 was analyzed using anti-CD54-PE or anti-CD86-PE. FITC, fluorescein isothiocyanate; FSC, forward scatter; PE, phycoerythrin; SSC, side scatter.

after collection. Biochemical and lymphocyte population characterizations were performed at the Department of Clinical Analysis and Department of Immunology of the "12 de Octubre" Hospital, respectively. Monocyte population and MV characterizations were conducted at the Department Genetics, Physiology, and Microbiology of Complutense University of Madrid (Spain). For MV characterization, plasma was obtained through centrifugation of blood samples at 1,500 × g for 20 min. Plasma samples were stored at −20°C.

Lymphocyte Characterization

Total lymphocytes, T lymphocytes (CD3+), T-helper lymphocytes (CD3+CD4+), T-cytotoxic lymphocytes (CD3+CD8+), B lymphocytes (CD3–CD19+), and natural killer (NK) cells (CD3–CD16+/CD56+) were analyzed (48, 49). Whole blood was stained using BD Multitest 6-color TBNK reagent (5:2 proportion; BD Biosciences, San José, CA, USA) for 15 min. Red blood cell lysis was performed using fluorescence-activated cell sorting (FACS) lysing solution (BD Biosciences). The lymphocyte subpopulations were determined using a FACSCanto II flow cytometer (BD Biosciences) and analyzed by the FACSCanto clinical software (BD Biosciences).

Monocyte Characterization

Classical (CD14++CD16–), intermediate (CD14++CD16+) and non-classical (CD14+CD16+) monocyte populations were

TABLE 1 | Baseline characteristics of patients and healthy subjects.

	HS	Patients with ACKD	Patients with KT
n	18	40	40
Age (years), mean ± SD[b]	51 ± 16	61 ± 17	54 ± 12
Male, *n* (%)[a]	9 (50%)	26 (65%)	27 (68%)
Etiopathology, *n* (%)[a]			
Nephroangiosclerosis	–	7 (17.5%)	6 (15%)
Diabetic nephropathy	–	13 (32.5%)	8 (20%)
Glomerular nephropathy	–	6 (15%)	4 (10%)
Polycystic kidney disease	–	4 (10%)	8 (20%)
Interstitial nephritis	–	6 (15%)	2 (5%)
Others	–	4 (10%)	10 (25%)
Hypertension, *n* (%)[a]	1 (6%)	36 (90%)***	39 (98%)***
Diabetes mellitus, *n* (%)[a]	2 (11%)	18 (45%)*	16 (40%)*
Dyslipidemia, *n* (%)[a]	0 (0%)	31 (78%)***	21 (53%)***#
Hyperuricemia, *n* (%)[a]	0 (0%)	28 (70%)***	13 (33%)***##
Smoking, *n* (%)[a]	4 (22%)	11 (28%)	10 (25%)
eGFR (mL/min/1.73 m^2), mean ± SD[d]	>90	16 ± 17***	49 ± 19***###
Serum creatinine (mg/dL), mean ± SD[c]	0.8 ± 0.2	4.2 ± 1.0***	1.5 ± 0.5***###
Serum albumin (mg/dL), mean ± SD[b]	4.7 ± 0.3	4.3 ± 0.4***	4.5 ± 0.4###
Proteins (mg/dL), mean ± SD[b]	7.1 ± 0.4	6.9 ± 0.5	7.0 ± 0.6
CRP (mg/dL), mean ± SD[d]	0.27 ± 0.5	0.45 ± 0.44**	0.47 ± 0.89*

*[a]Chi-squared test. [b]ANOVA (Tukey test). [c]ANOVA (Games–Howell test). [d]Mann–Whitney U-test. Statistical significance was denoted by *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 vs. HS; #p ≤ 0.05, ##p ≤ 0.01, ###p ≤ 0.001 vs. ACKD.*

ACKD, advanced chronic kidney disease; ANOVA, analysis of variance; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HS, healthy subjects; KT, kidney transplantation; SD, standard deviation.

analyzed as previously described (50) with modifications. In addition, the expression of CD86/B-lymphocyte antigen B7-2 (CD86/B7-2) and CD54/intercellular adhesion molecule 1 (CD54/ICAM1) in each population was determined. A triple-staining immunofluorescence technique was utilized, and flow cytometry analysis was performed. Monoclonal antibodies conjugated with fluorochromes against CD14 (TuK4 clone, TRI-COLOR®; Invitrogen, Carlsbad, CA, USA), CD16 (3G8 clone, fluorescein isothiocyanate [FITC]; Invitrogen), CD86/B7.2 (BU63 clone, phycoerythrin; Biolegend, San Diego, CA, USA), and CD54/ICAM1 (MEM-111 clone, phycoerythrin; Invitrogen) were used.

Briefly, whole blood was incubated with the corresponding antibody for 25 min at room temperature in darkness. Red blood lysis was performed using FACS Lysing Solution (BD Biosciences) for 10 min prior to centrifugation at 400 × g. The cells were fixed using Cell Fix (BD Biosciences) and stored at 4°C until assessment. The maximum storage period was 24 h. The monocyte subpopulations and phenotypes were determined using a FACSCalibur cytometer (BD Biosciences), with the support of the staff of the cytometry associated research center of Complutense University of Madrid (Spain) and analyzed by the FlowJo™ software (Ashland, OR, USA). The results were expressed as the percentage of monocyte subtype with respect to the total monocyte population, in the case of three subtypes of monocytes. Alternatively, data were presented as the percentage of each population that expressed CD86/CD54 and the mean fluorescence intensity (MFI), which represents the amount of molecule expressed by each monocyte (**Figure 2**).

MV Characterization

The total number of MVs (AnnexinV+) and endothelial-derived MVs (AnnexinV+CD31+CD41–), as well as the expression of tissue factor (CD142) in endothelial-derived MVs, were determined as previously described (49). A quadruple-staning immunofluorescence technique was utilized, and flow cytometry analysis was performed. Monoclonal antibodies conjugated with fluorochromes against Annexin V (Annexin V-FITC Kit; Miltenyi Biotec, Bergisch Gladbach, Germany), CD41/integrin subunit alpha 2b (MEM-06 clone, peridnine chlorophyll protein; Invitrogen), CD31/platelet and endothelial cell adhesion molecule 1 [PECAM1] (WM-59 clone, phycoerythrin; BD Bioscience), and CD142/tissue factor (HTF-1 clone, allophycocyanin [APC]; Invitrogen) were used.

Briefly, platelet-free plasma samples were centrifuged at 110,000 × g for 2 min and resuspended in Annexin-V binding buffer (Annexin V-FITC Kit; Miltenyi Biotec). Subsequently, the samples were incubated with the corresponding antibodies for 40 min at room temperature in darkness, fixed using Cell Fix (BD Bioscience), and stored at 4°C until assessment. The

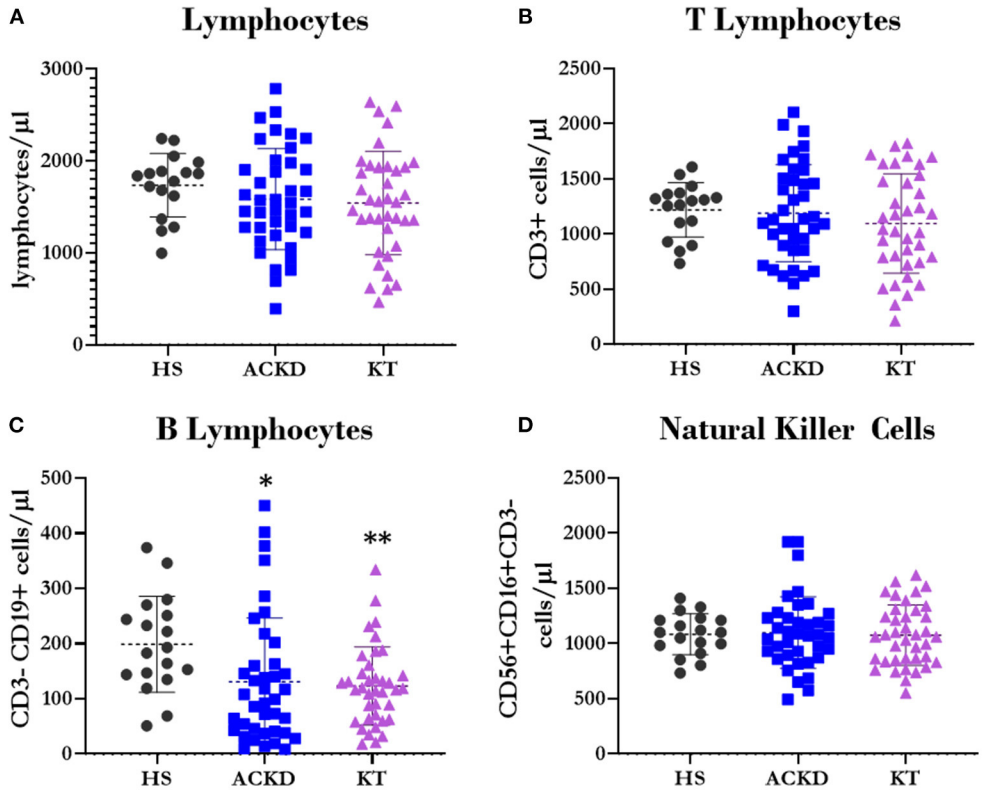


FIGURE 3 | Description of lymphocyte subpopulations. Number of total lymphocytes$^{\Psi}$ **(A)**, T lymphocytes$^{\Omega}$ **(B)** (CD3+), B lymphocytes$^{\Omega}$ **(C)** (CD3–CD19+), and natural killer cells$^{\Psi}$ **(D)** (CD56+CD16+CD3–) in healthy subjects (HS), patients with advanced chronic kidney disease (ACKD), and patients with kidney transplantation (KT). $^{*}p \leq 0.05$, $^{**}p \leq 0.01$ vs. HS. Statistical analysis: $^{\Psi}$ANOVA (Tukey test). $^{\Omega}$ANOVA (Games–Howell test). ANOVA, analysis of variance.

maximum storage period was 24 h. The MV subpopulations were characterized through flow cytometry using a FACSCalibur cytometer (BD Biosciences) with the support of the staff of the cytometry associated research center of Complutense University of Madrid (Spain) and analyzed by the FlowJo™ software. The standardization on the FACSCalibur device was carried out as previously described (49).

Statistical Analysis

SPSS version 21.0 (Armonk, NY, USA) was used for the statistical analysis. The data were expressed as the mean ± standard deviation. The normality of the samples and variance homogeneity were checked using one-sample Kolmogorov–Smirnov and Levene tests. Normal variables were evaluated using one-way analysis of variance to determine individual differences for each parameter followed by *post-hoc* analysis. The *post-hoc* analysis was performed using Tukey's test for variables with homogeneous variances and the Games–Howell test for those with heterogeneous variances. For non-normal variables, the Mann–Whitney *U*-test was performed. For qualitative data, the chi-squared test was performed, and the results were expressed as relative and absolute frequencies. The Spearman correlation test was carried out for correlation analysis between lymphocytes, monocytes, and MV subpopulations in renal transplant patients. $P \leq 0.05$ denoted statistical significance.

RESULTS

Population Description

The baseline characteristics of patients with CKD and HS are shown in **Table 1**. There was no difference between the age or sex of patients with ACKD (61 ± 17 years; 65% males) and transplantation (54 ± 12 years; 68% males). The numbers of individuals affected by hypertension and diabetes mellitus were similar in both groups of patients (ACKD: 90% and 45%; KT: 98% and 40%, respectively) as well as the smoking habit (ACKD: 28%; KT: 25%). Whereas de number of patients with dyslipidemia and hyperuricemia was higher in ACKD (78%, $p = 0.034$; 70%, $p = 0.002$, respectively) than in kidney transplantation (53; 33%, respectively). The estimated GFR was lower in patients with ACKD (16 ± 17 mL/min/1.73 m^2, $p = 0.000$) than in those with kidney transplantation (49 ± 19 mL/min/1.73 m^2). Moreover, there were no differences in the levels of C-reactive protein in both patient groups (ACKD: 0.45 ± 0.44 mg/dL; KT: 0.47 ± 0.89 mg/dL).

Regarding the immunosuppressive treatment, the most used treatment was a combination of tacrolimus and mycophenolic

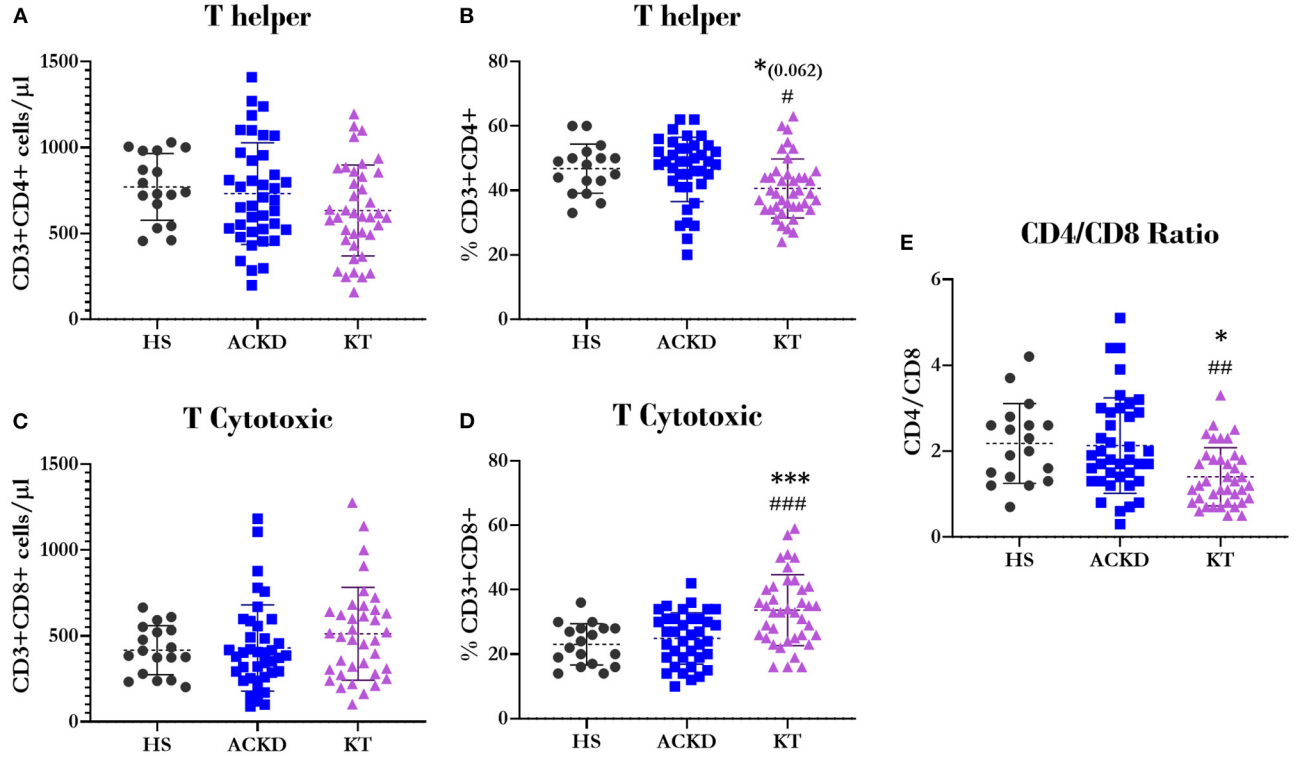


FIGURE 4 | Description of T lymphocyte subpopulations. Number$^{\Psi}$ **(A)** and percentage$^{\Psi}$ **(B)** of T-helper lymphocytes (CD3+CD4+); number$^{\Psi}$ **(C)** and percentage$^{\Psi}$ **(D)** of T-cytotoxic lymphocytes (CD3+CD8+); relationship between helper and cytotoxic lymphocytes$^{\Omega}$ **(E)** (CD4/CD8 ratio) in healthy subjects (HS), patients with advanced chronic kidney disease (ACKD), and patients with kidney transplantation (KT). $^{*}p \leq 0.05$, $^{***}p \leq 0.001$ vs. HS; $^{\#}p \leq 0.05$, $^{\#\#}p \leq 0.01$, and $^{\#\#\#}p \leq 0.001$ vs. ACKD. Statistical analysis: $^{\Psi}$ANOVA (Tukey test). $^{\Omega}$ANOVA (Games–Howell test). ANOVA, analysis of variance.

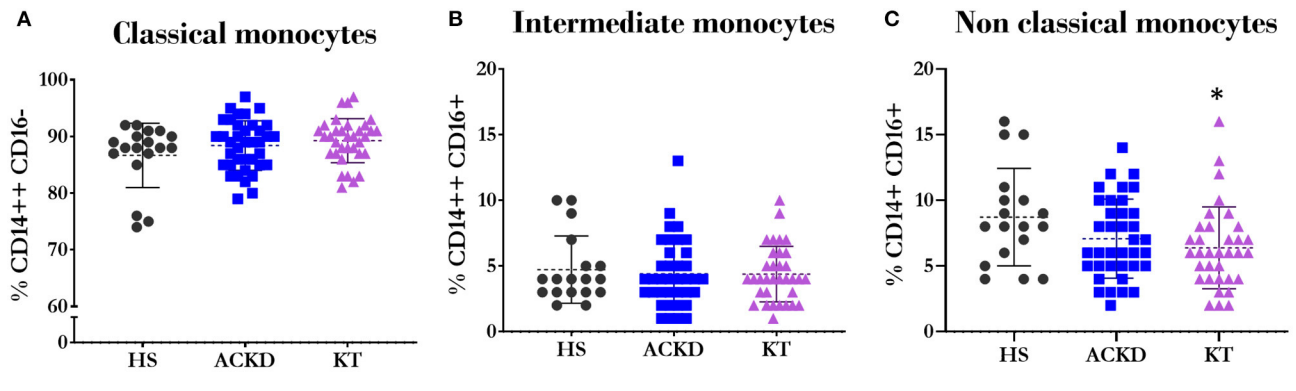


FIGURE 5 | Description of monocyte subsets. Percentage of classical$^{\Phi}$ **(A)** (CD14++CD16−); intermediate$^{\Psi}$ **(B)** (CD14++CD16+) and non-classical$^{\Psi}$ **(C)** (CD14+CD16+) monocytes in healthy subjects (HS), patients with advanced chronic kidney disease (ACKD), and patients with kidney transplantation (KT). $^{*}p \leq 0.05$ vs. HS. Statistical analysis: $^{\Psi}$ANOVA (Tukey test). $^{\Psi}$Mann–Whitney *U*-test. ANOVA, analysis of variance.

acid (26 patients, 65%), followed by a combination of tacrolimus and everolimus (seven patients, 17.5%).

Lymphocyte Characterization

The present results did not show any differences in the total number of lymphocytes (**Figure 3A**), T lymphocytes (**Figure 3B**), or NK cells (**Figure 3D**). A decrease in the number of B lymphocytes was observed in ACKD (130.86 ± 155.55 cells/µL) and KT (123.05 ± 71.07 cells/µL) patients vs. HS (198.77 ± 87.08 cells/µL, $p = 0.047$ and 0.009, respectively) (**Figure 3C**).

Regarding the T-lymphocyte subpopulations, we did not find differences in the total numbers of T-helper and T-cytotoxic cells (**Figures 4A,C**, respectively). Nevertheless, patients with KT showed a decrease in the percentage of T-helper lymphocytes

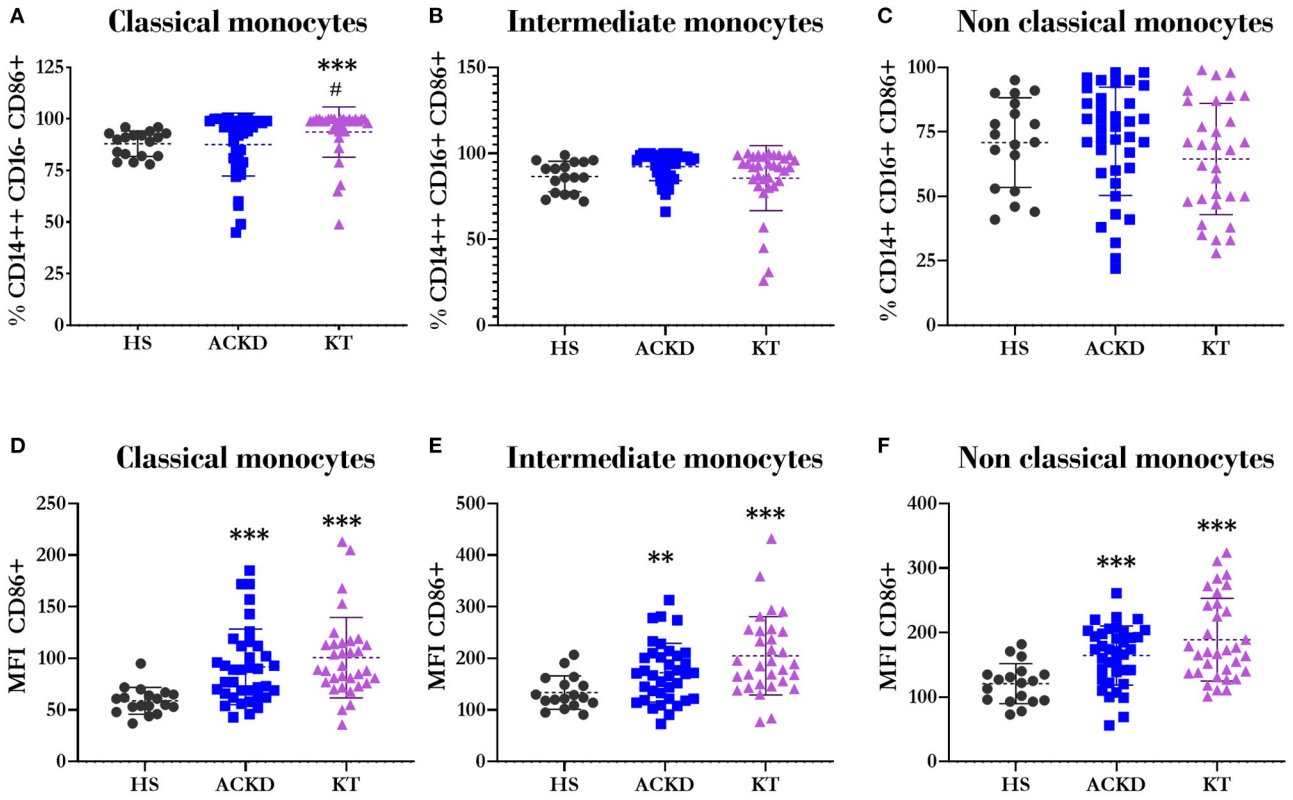


FIGURE 6 | Expression of CD86/B7.2 in different monocyte subsets. Percentage of classical$^{\Phi}$ **(A)** (CD14++CD16–), intermediate$^{\Omega}$ **(B)** (CD14++CD16+), and non-classical$^{\Psi}$ **(C)** (CD14+CD16+) monocytes expressing CD86. Mean fluorescence intensity of CD86 in classical$^{\Omega}$ **(D)** (CD14++CD16–), intermediate$^{\Omega}$ **(E)** (CD14++CD16+), and non-classical$^{\Omega}$ **(F)** (CD14+CD16+) monocytes in healthy subjects (HS), patients with advanced chronic kidney disease (ACKD), and patients with kidney transplantation (KT). $^{**}p \leq 0.01$, $^{***}p \leq 0.001$ vs. HS; $^{\#}p \leq 0.05$ vs. ACKD. Statistical analysis: $^{\Psi}$ANOVA (Tukey test). $^{\Omega}$ANOVA (Games–Howell test). $^{\Phi}$Mann–Whitney *U*-test. ANOVA, analysis of variance.

(40.62 ± 9.16%, $p = 0.062$ vs. HS and $p = 0.016$ vs. ACKD; **Figure 4B**). They also exhibited an increase in the cytotoxic subpopulation (33.67 ± 11.01%, $p = 0.000$; **Figure 4D**) compared with HS (46.76 ± 7.64 and 23.06 ± 6.41%, respectively) and patients with ACKD (46.51 ± 9.96 and 24.89 ± 7.97%, respectively), resulting in a decreased CD4/CD8 ratio (KT: 1.4 ± 0.68, HS: 2.18 ± 0.93, $p = 0.011$; ACKD: 2.13 ± 1.11, $p = 0.002$; **Figure 4E**).

Monocyte Characterization

There were no differences between groups in the percentages of classical (**Figure 5A**) and intermediate (**Figure 5B**) monocytes. Patients with KT had a lower percentage of non-classical monocytes (6.38 ± 3.11%) (**Figure 5C**) compared with HS (8.72 ± 3.7%, $p = 0.036$).

However, the most notable differences were observed in CD86 and CD54 in different subpopulations. There was a higher percentage of classical monocytes that express CD86 in KT patients (93.63 ± 11.99%) vs. HS (88 ± 6.15%, $p = 0.000$) and ACKD patients (87.92 ± 15.15%, $p = 0.03$; **Figure 6A**). Notably, the percentage of monocytes expressing this molecule did not change in the intermediate (**Figure 6B**) and non-classical (**Figure 6C**) subtypes. Meanwhile, the number of cells expressing CD86 was increased in patients with ACKD (classical: 91.76 ± 36.68 MFI, $p = 0.000$; intermediate: 172.21 ± 56.94 MFI, $p = 0.008$; non-classical: 164.43 ± 45.79 MFI, $p = 0.000$) and those with KT (classical: 100.7 ± 38.97 MFI, $p = 0.000$; intermediate: 208.88 ± 78.01 MFI, $p = 0.000$; non-classical: 188.97 ± 64.18 MFI, $p = 0.000$) compared with HS (classical: 58.94 ± 12.98 MFI; intermediate: 133.76 ± 32.41 MFI; non-classical: 120.78 ± 31 MFI) in the three monocyte subpopulations (**Figures 6D–F**).

Regarding the expression of CD54 in the different subsets of monocytes, there was an increase in the percentage of classical monocytes expressing this molecule in patients with KT (96.13 ± 4.49%) vs. HS (89 ± 6.18%, $p = 0.001$) and patients with ACKD (85.95 ± 13.25%, $p = 0.000$; **Figure 7A**). However, there was no difference in the percentage of intermediate (**Figure 7B**) and non-classical monocytes (**Figure 7C**). The expression level of CD54 in the three subsets (**Figures 7D–F**) was higher in patients with KT (classical: 167.9 ± 66.43 MFI; intermediate: 316.6 ± 100.41 MFI; non-classical: 210 ± 64.06 MFI) compared with HS (classical: 123.11 ± 30.55 MFI, $p = 0.008$; intermediate: 245.18 ±

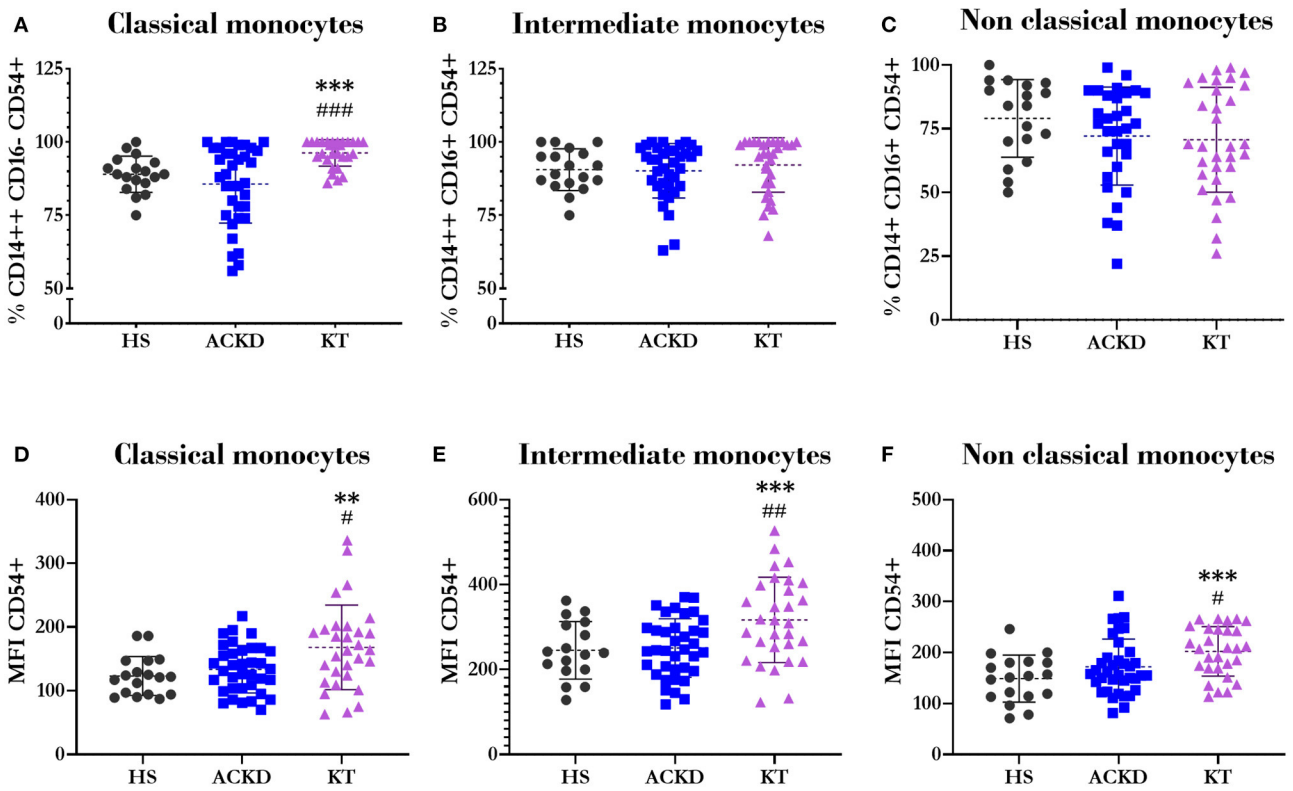


FIGURE 7 | Expression of CD54/ICAM1 in different monocyte subsets. Percentage of classical$^{\Omega}$ **(A)** (CD14++CD16−), intermediate$^{\Psi}$ **(B)** (CD14++CD16+), and non-classical$^{\Psi}$ **(C)** (CD14+CD16+) monocytes expressing CD54. Mean fluorescence intensity of CD54 in classical$^{\Omega}$ **(D)** (CD14++CD16−), intermediate$^{\Psi}$ **(E)** (CD14++CD16+), and non-classical$^{\Psi}$ **(F)** (CD14+CD16+) monocytes in healthy subjects (HS), patients with advanced chronic kidney disease (ACKD), and patients with kidney transplantation (KT). $^{**}p \leq 0.01$, $^{***}p \leq 0.001$ vs. HS; $^{\#}p \leq 0.05$, $^{\#\#}p \leq 0.01$, $^{\#\#\#}p \leq 0.001$ vs. ACKD. Statistical analysis: $^{\Psi}$ANOVA (Tukey test). $^{\Omega}$ANOVA (Games-Howell test). ANOVA, analysis of variance.

67.73, $p = 0.014$; non-classical: 148.78 ± 46.05 MFI, $p = 0.001$), and patients with ACKD (classical: 133.67 ± 37.29 MFI, $p = 0.041$; intermediate: 250.51 ± 68.92 MFI, $p = 0.004$; non-classical: 171.92 ± 54.24 MFI, $p = 0.02$).

MVs Characterization

The total numbers of MVs (**Figure 8A**) and endothelial MVs (**Figure 8B**) were increased in patients with ACKD (94,335.97 ± 124,672 MVs/μL; 66,355.47 ± 124,672.09 MVs/μL; respectively) compared with HS (8,599.14 ± 5,341.19 MVs/μL, $p = 0.001$; 7,417.7 ± 11,418.63 MVs/μL, $p = 0.02$; respectively) and patients with KT (12,286.87 ± 11,637.93 MVs/μL, $p = 0.001$; 6,412.73 ± 764.68 MVs/μL, $p = 0.001$; respectively). There were no differences observed in the percentage of endothelial MVs (**Figure 8C**). The percentage of endothelial MVs expressing tissue factor (CD142) (**Figure 8D**) was higher in patients with ACKD (8,327.29 ± 1,736.99%) vs. HS (664.29 ± 703.9%, $p = 0.003$) and patients with KT (845.76 ± 1,390%, $p = 0.000$). A lower number of endothelial MVs expressing tissue factor (**Figure 8E**) was observed in patients with KT (128.78 ± 139.2 MVs/μL) compared with HS (153.5 ± 151.55 MVs/μL, $p = 0.017$) and patients with ACKD (378.68 ± 315.89 MVs/μL, $p = 0.000$).

Correlations

The correlations between the subpopulations of lymphocytes, monocytes, and MVs in patients with KT are shown in **Figures 9–13**.

The total number of lymphocytes showed a positive correlation with the percentage ($r = 0.376$, $p = 0.041$; **Figure 9A**) and expression ($r = 0.339$, $p = 0.062$ statistical trend; **Figure 9B**) of CD86 and percentage ($r = 0.589$, $p = 0.001$; **Figure 9C**) and expression ($r = 0.421$, $p = 0.026$; **Figure 9D**) of CD54 in non-classical monocytes in all cases.

Regarding T lymphocytes, we observed a positive correlation with the expression of CD86 in non-classical monocytes ($r = 0.430$, $p = 0.018$; **Figure 9E**), the percentage of classical ($r = 0.430$, $p = 0.018$; **Figure 9F**), intermediate ($r = 0.471$, $p = 0.011$; **Figure 9G**) and non-classical ($r = 0.494$, $p = 0.008$, **Figure 9H**) CD54+ monocytes, and the expression of CD54 in non-classical monocytes ($r = 0.363$, $p = 0.063$ statistical trend, **Figure 9I**).

We found a positive correlation between B cells and monocytes in the percentage of intermediate ($r = 0.413$, $p = 0.017$; **Figure 10A**) and non-classical ($r = 0.323$, $p = 0.067$; **Figure 10B**) monocytes, intermediate ($r = 0.433$, $p = 0.013$; **Figure 10C**) and non-classical ($r = 0.354$, $p = 0.051$; **Figure 10D**) monocytes that express CD86, and the expression of CD86 in

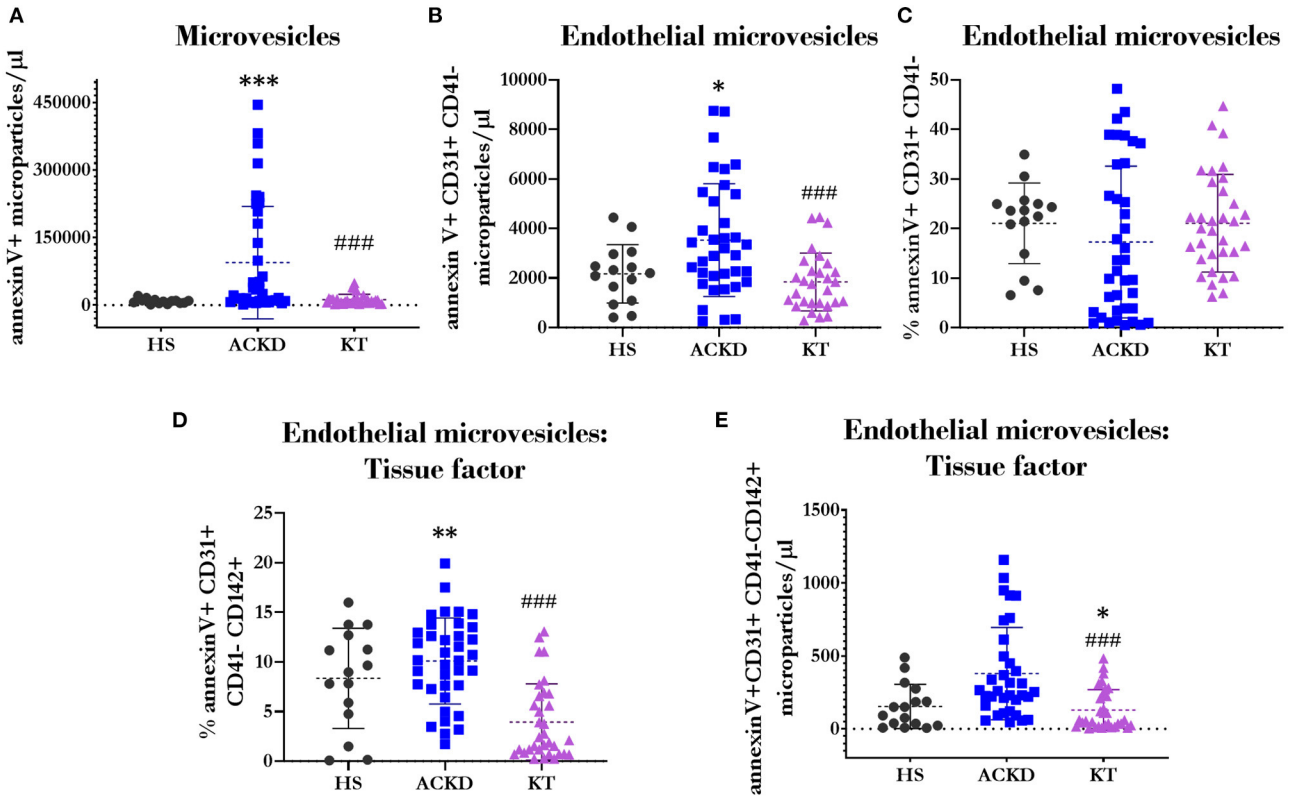


FIGURE 8 | Description of the microvesicles phenotype. Total number of microvesicles$^{\Phi}$ **(A)** (annexin V+); number$^{\Omega}$ **(B)** and percentage$^{\Omega}$ **(C)** of endothelial microvesicles (annexin V+CD31+CD41–); and percentage$^{\Psi}$ **(D)** and number$^{\Phi}$ **(E)** of endothelial microvesicles expressing tissue factor (CD142) in healthy subjects (HS), patients with advanced chronic kidney disease (ACKD), and patients with kidney transplantation (KT). $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, and $^{***}p \leq 0.001$ vs. HS; $^{\#\#\#}p \leq 0.001$ vs. ACKD. Statistical analysis: $^{\Psi}$ANOVA (Tukey test). $^{\Omega}$ANOVA (Games–Howell test). $^{\Phi}$Mann–Whitney *U*-test. ANOVA, analysis of variance.

classical ($r = 0.446$, $p = 0.010$; **Figure 10E**) and intermediate ($r = 0.371$, $p = 0.040$; **Figure 10F**) monocytes.

There was a positive correlation between the number of NK cells and the percentage of non-classical monocytes that expressed CD86 ($r = 0.349$, $p = 0.054$; **Figure 10G**), the expression of CD86 in classical ($r = 0.401$, $p = 0.023$; **Figure 10H**), intermediate ($r = 0.461$, $p = 0.009$; **Figure 10I**), and non-classical ($r = 0.469$, $p = 0.007$; **Figure 10J**) monocytes, and the percentage of non-classical monocytes that expressed CD54 ($r = 0.472$, $p = 0.009$; **Figure 10K**).

Regarding the number of T-cytotoxic lymphocytes, we observed a positive correlation with the percentage of classical ($r = 0.456$, $p = 0.013$; **Figure 11A**) and non-classical ($r = 0.453$, $p = 0.012$; **Figure 11B**) monocytes that expressed CD54, and the expression of CD54 in classical ($r = 0.351$, $p = 0.062$; **Figure 11C**) and intermediate ($r = 0.369$, $p = 0.049$; **Figure 11D**) monocytes.

With respect to the relationship between lymphocytes and MVs, the percentage of T-helper lymphocytes was negatively correlated with the number ($r = -0.500$, $p = 0.006$; **Figure 12A**) and percentage ($r = -0.364$, $p = 0.037$; **Figure 12B**) of endothelial MVs, and positively correlated with the percentage of endothelial MVs ($r = 0.588$, $p = 0.000$; **Figure 12C**). CD4/CD8 was negatively correlated with the number ($r = -0.429$, $p = 0.023$; **Figure 12D**) and percentage ($r = -0.588$, $p = 0.000$; **Figure 12E**) of endothelial MVs.

Finally, the percentage of non-classical monocytes expressing CD86 was negatively correlated with the total number of MVs ($r = -0.447$, $p = 0.025$; **Figure 13A**). Moreover, there was a negative correlation between the expression of CD86 in classical monocytes and the percentage of endothelial MVs expressing tissue factor ($r = -0.440$, $p = 0.025$; **Figure 13B**). Of note, the expression of CD86 in intermediate ($r = 0.378$, $p = 0.062$; **Figure 13C**) and non-classical ($r = 0.378$, $p = 0.057$; **Figure 13D**) monocytes was positively correlated with the number of total MVs. There was a negative correlation between the percentage of intermediate monocytes expressing CD54 and the total number of MVs ($r = -0.448$, $p = 0.028$; **Figure 13E**) and endothelial MVs ($r = -0.458$, $p = 0.037$; **Figure 13F**).

DISCUSSION

In this cross-sectional study, we analyzed the immune phenotype of lymphocytes, monocytes, and MVs in patients with ACKD and KT vs. HS. The patients with KT showed B-cell lymphopenia, an increased proportion of T-cytotoxic lymphocytes, and increased

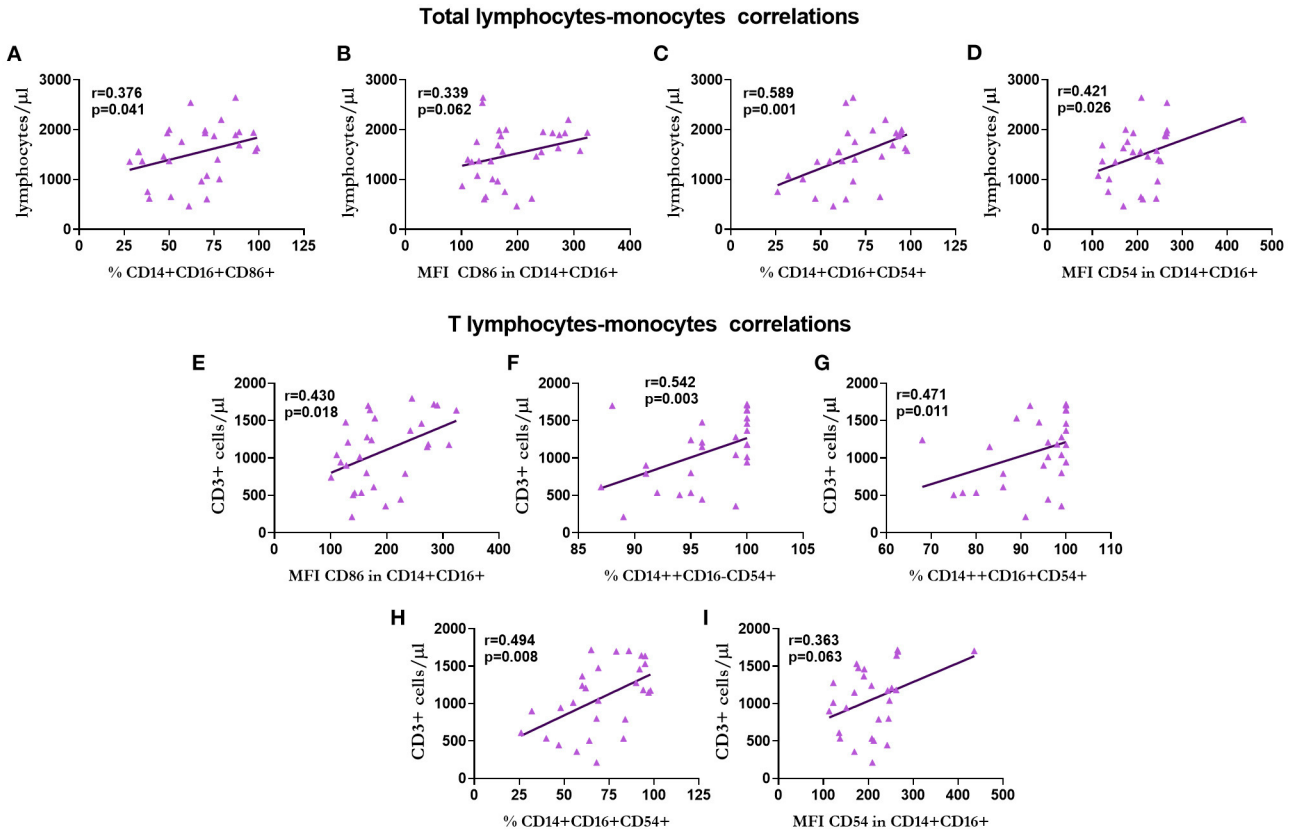


FIGURE 9 | Correlation between the number of total lymphocytes and T lymphocytes with monocytes in renal transplantation. Correlation of the total number of lymphocytes with the percentage of non-classical monocytes CD86+ **(A)**, expression of CD86 in non-classical monocytes **(B)**, percentage of non-classical monocytes expressing CD54 **(C)**, and expression of CD54 in non-classical monocytes **(D)**. Correlations of T lymphocytes with the expression of CD86 in non-classical monocytes **(E)**, percentage of classical **(F)**, intermediate **(G)**, and non-classical **(H)** CD54+ monocytes, and the expression of CD54 in non-classical monocytes **(I)** are shown.

levels of adhesion (CD54) and co-stimulatory (CD86) molecules in all monocyte subsets. Furthermore, the changes in lymphocyte subpopulations were positively correlated with the monocyte phenotypes, and both types of leukocytes were negatively correlated with changes in the MV phenotype. This is the first study that investigated the correlations between changes in lymphocytes, monocytes, and MVs. Although those changes could be directly or indirectly influenced by immunosuppressive treatment, the characteristic of those cells and molecules could participate in the development of cardiovascular and renal complications that persisted in patients with KT.

Currently, KT is the best therapy for CKD; however, patients require immunosuppressive treatment to avoid allograft rejection. The treatment may differ between patients due to numerous factors, such as the immunological risk for rejection, nutritional status, and the presence of other co-morbidities (51). Most patients with KT receive different immunosuppressive therapies that seek a balance to avoid acute rejection, toxicity (52), and possible deleterious effects, such as infections (53) and tumors (54). Most patients undergoing renal transplantation receive a combination (two or more) of calcineurin inhibitors (tacrolimus), azathioprine, mycophenolic acid, mammalian target of rapamycin (mTOR)-inhibitors, prednisone, and belatacept (51, 55–58).

The main objective of immunosuppressive treatment is the regulation of the T cell-mediated alloimmune response (51), which is induced by the response of the immune system to non-self-antigens of the same species. In this process, T cells play an essential role in recognizing the non-self-antigen in the context of the major histocompatibility complex (59, 60). Therefore, most immunosuppressors inhibited the activation of T cells and avoided the proliferation of activated B, T, and NK cells due to alteration in the synthesis of cytokines (61, 62). The present findings did not show changes in the total number of T lymphocytes and NK cells, whereas B-cell lymphopenia was noted in both groups of patients. Thus far, only a few studies have measured the total number of lymphocytes or the total number of T lymphocytes, without reporting any differences (63, 64). Other studies also showed the presence of B-cell lymphopenia in patients with ACKD (65, 66) and KT. Meanwhile, in ACKD, this diminution may be associated with a decrease in GFR. Nevertheless, in renal transplantation, the effect of treatment on GFR remains unclear. Some studies reported an increase in the number of NK cells (63); however, the changes in NK

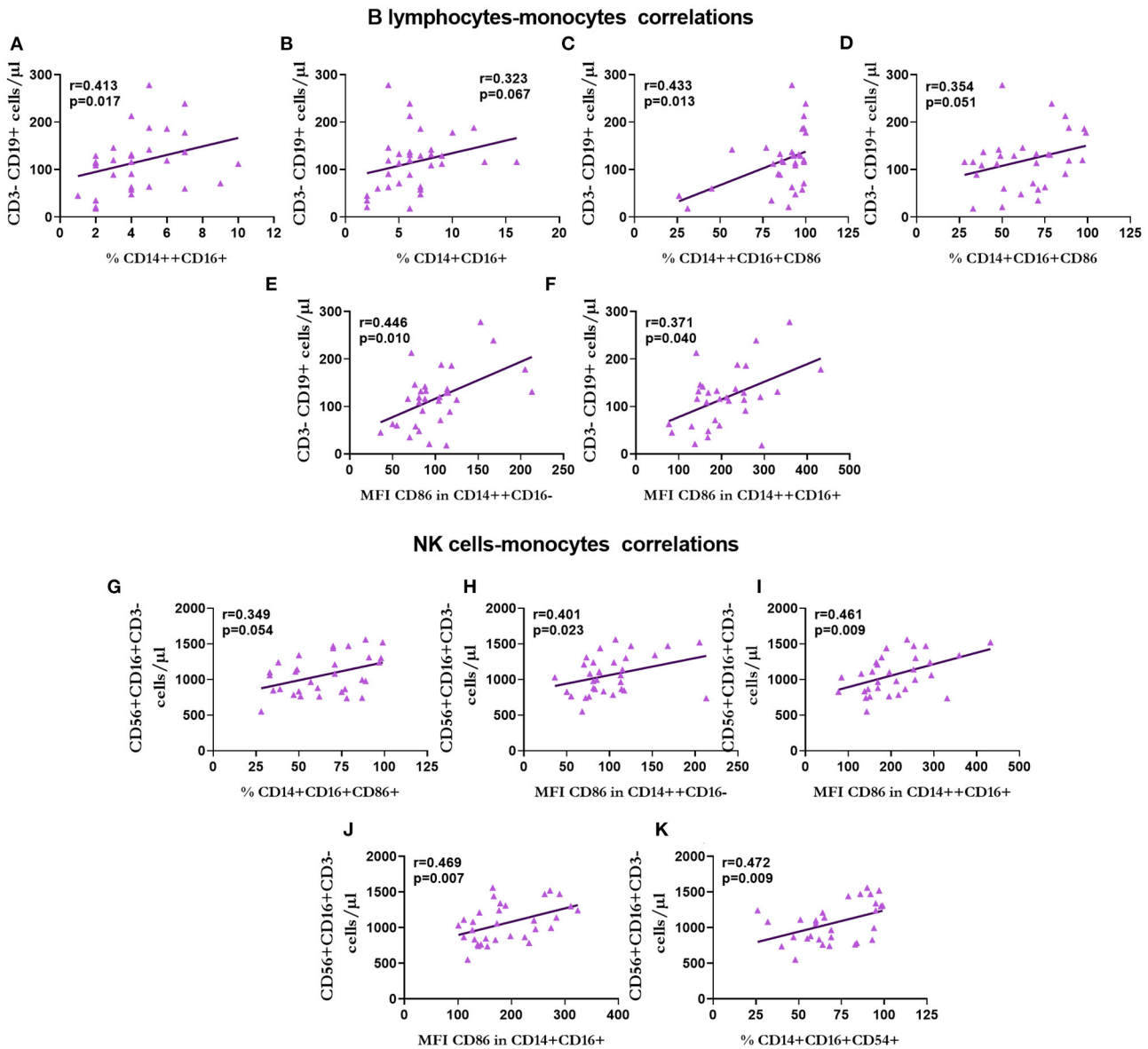


FIGURE 10 | Correlation between the number of B lymphocytes and natural killer (NK) cells with monocytes in renal transplantation. Correlation between B lymphocytes and the percentage of intermediate **(A)** and non-classical **(B)** monocytes, the percentage of intermediate **(C)** and non-classical **(D)** CD86+ monocytes and the expression of CD86 in classical **(E)** and intermediate **(F)** monocytes. Correlations of NK cells with the percentage of CD86+ monocytes **(G)**, expression of CD86 in classical **(H)**, intermediate **(I)**, and non-classical **(J)** monocytes, and the percentage of non-classical monocytes expressing CD54 **(K)** are shown.

cells appeared to depend on the immunosuppressor treatment (64, 67).

Changes in the T-helper and T-cytotoxic subpopulations have been more widely investigated. The majority of the research studies, similar to the present investigation, did not report differences in the number of T subpopulations. Instead, they reported an increase in the proportion of the T-cytotoxic subpopulation compared with that of T-helper cells (63, 64). The regulation of T-helper cells may play a key role in the prevention of negatives outcomes in patients undergoing renal transplantation. Persistent CD4+ lymphopenia has been related to atherosclerosis (68) and an increase in morbidity and mortality in patients with KT (69).

Changes in monocyte subsets in renal transplantation have not been thoroughly studied. Intermediate (CD14++CD16+) and non-classical monocytes exhibited pro-inflammatory and proatherogenic activities (CD14+CD16+) in health individuals and in patients with CKD (4, 32, 70, 71). Some studies showed a depletion of non-classical monocytes due to treatment with glucocorticoids (72–74). The wide use of corticoids in immunosuppression may explain the decrease in non-classical monocytes recorded in the renal transplantation group.

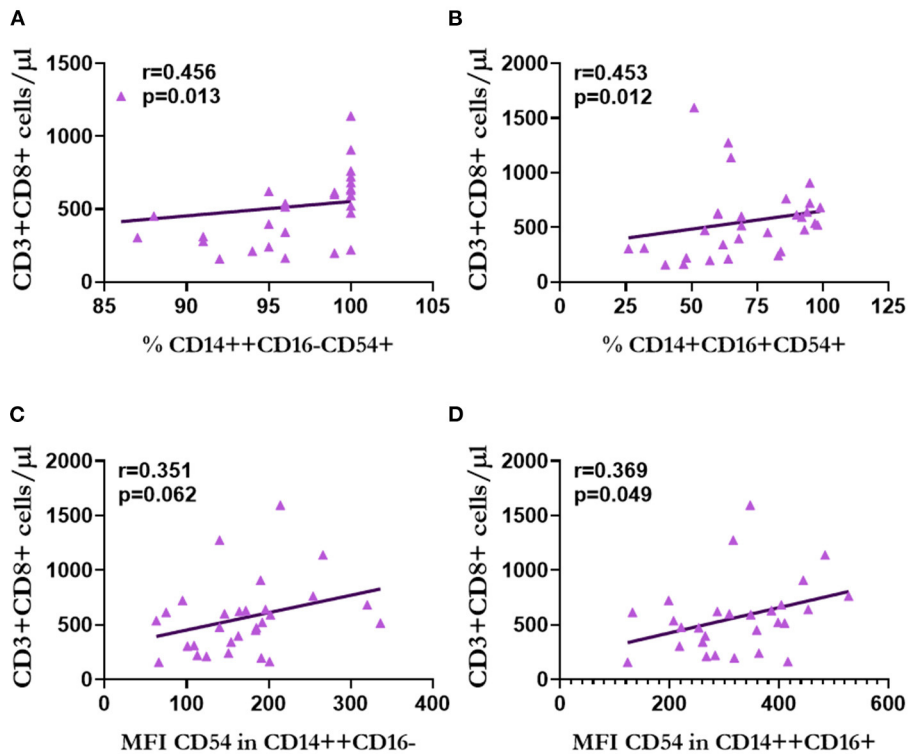


FIGURE 11 | Correlation between T-cytotoxic lymphocytes and monocytes in renal transplantation. Correlations between T-cytotoxic lymphocytes and the percentage of classical **(A)** and non-classical **(B)** CD54+ monocytes, and the expression of CD54 in classical **(C)** and intermediate **(D)** monocytes are shown.

There is limited research on the expression of CD86/B7.2 and CD54/ICAM1 in the monocyte subsets, particularly in CKD. CD80/b7.1 and CD86/B7.2 are co-stimulatory molecules, which are essential for the activation of T cells. This co-stimulation is exhibited by the antigen-presenting cells. Some studies did not report changes in the expression of CD86 in monocytes of patients with chronic renal failure (75), whereas others showed a decrease in its expression in monocytes (75) and dendritic cells (76) of patients undergoing dialysis. Nevertheless, the proinflammatory and proatherogenic monocytes showed an increase in CD86 expression (77, 78).

Although further research is warranted, the microinflammatory state of the CKD transplant could lead to the development of senescent monocytes with an increased expression of CD86, explaining the present results. Regarding the expression of CD86 by monocytes in patients with KT, the blockage of B7/CD28 co-stimulation required a specific antibody against B7 components (79–81). This is rarely used and had shown more significant effect but differs between the two subtypes of B7 due to differences in biochemical characteristics (82, 83). CD54/ICAM1 is an adhesion molecule expressed by immune and endothelial cells. The increased expression of ICAM1 in allograft tissue is related to rejection (84, 85). The monocytes of patients who underwent transplantation and were treated with mycophenolate mofetil did not show any differences in the expression of CD54 (86). The expression of CD86 and CD54 is markedly increased in intermediate and non-classical monocytes (4, 87, 88). These monocytes are highly proinflammatory and participate in atherosclerosis (4). The elevation in the expression of these molecules in all monocyte subsets of patients with transplantation may indicate an increase in senescent monocytes participating in cardiovascular disease, which is one of the main causes of death in patients with KT (89). The increase on the expression in costimulatory molecules has been shown in autoimmune disease; in particular, a increase of these costimulatory molecules in monocytes and in plasma lead to dysregulation of the immune response toward an exacerbate inflammatory one (90–92).

It was recently discovered that MVs are a form of extracellular communication. They play an essential role in the development of multiples disease (93, 94), but they have been extensively studied in cardiovascular alterations (95–98). In disease, there is an increase in the number and changes in the content of MVs (96). The increase in indoxyl sulfate shown in CKD has been related to the increase in endothelial MVs that participated in vascular calcification (98, 99). This increase in indoxyl sulfate and other uremic toxins may explain the increased number of MVs and endothelial MVs in patients with ACKD. Transplantation

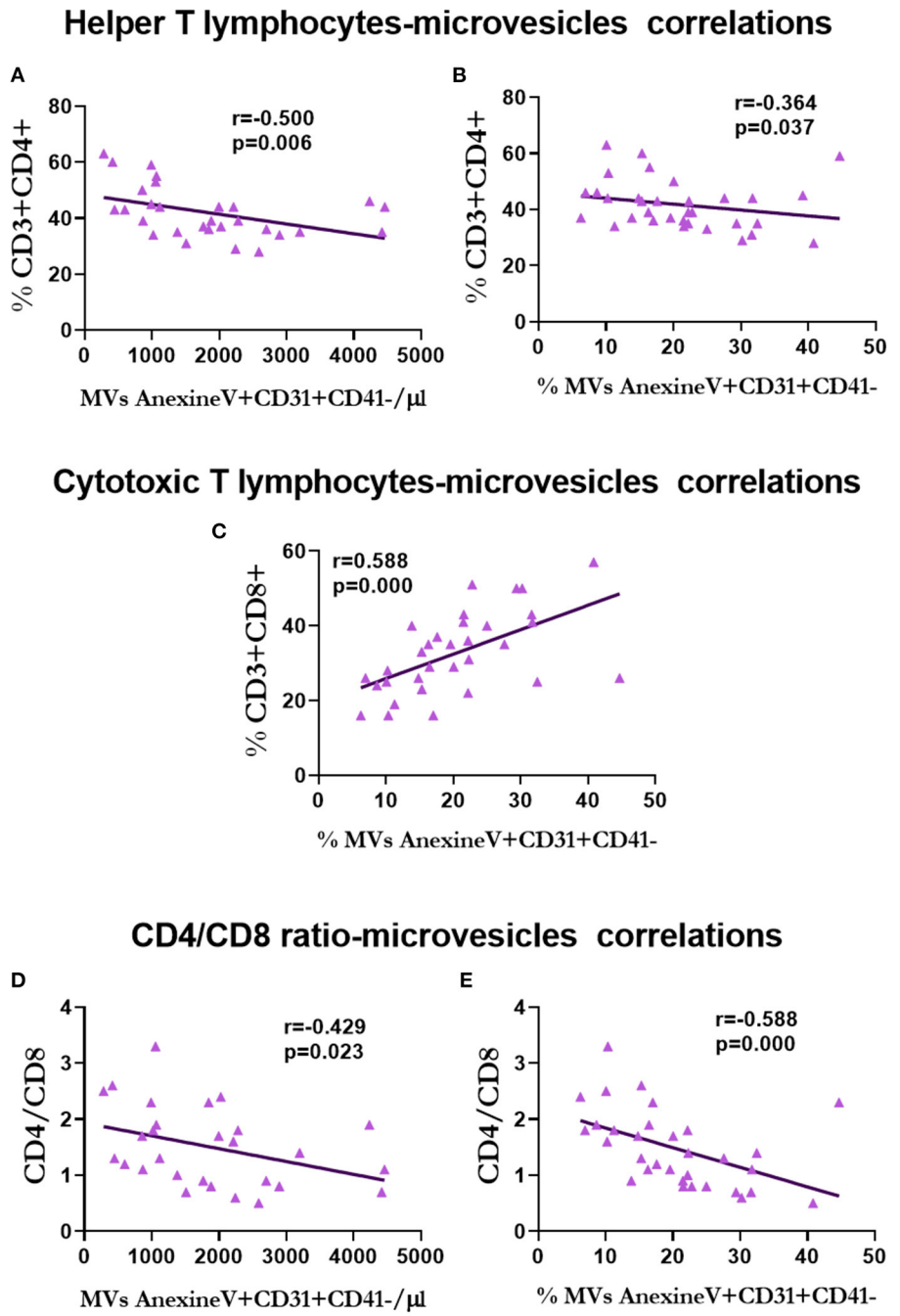


FIGURE 12 | Correlation between lymphocytes and microvesicles in renal transplantation. Correlation between T-helper lymphocytes and the number **(A)** and percentage **(B)** of endothelium microvesicles. Correlation between T-cytotoxic lymphocytes and percentage of endothelium microvesicles **(C)**. Correlation of CD4/CD8 ratio with the number **(D)** and percentage **(E)** of endothelium microvesicles.

partially solves this problem by increasing kidney function. Tissue factor (CD142) triggers thrombotic responses and plays an important role in atherosclerosis. Thus, elevated levels of tissue factor in microparticles is associated with an increased risk of atherosclerosis and thrombosis (100–102). The elevation in the expression of tissue factor in patients with ACKD contributes to the increased risk of cardiovascular disease in patients with CKD.

To the best of our knowledge, this is the first study to correlate changes in lymphocyte subsets with different monocyte

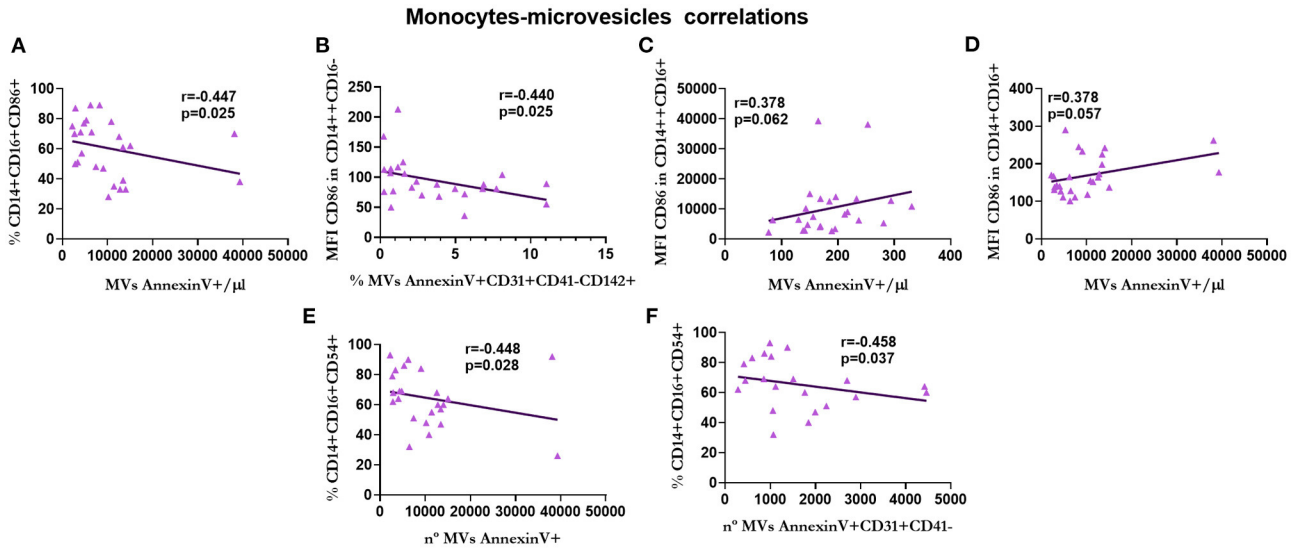


FIGURE 13 | Correlation between monocytes and microvesicles in renal transplantation. Correlations between the percentage of CD86+ non-classical monocytes and the total number of microvesicles **(A)**, the expression of CD86 in classical monocytes and the percentage of 142+ endothelium microvesicles **(B)**, the expression of CD86 in intermediate monocytes and the total number of microvesicles **(C)**, the expression of CD86 in non-classical monocytes and the total number of microvesicles **(D)**, the percentage of CD54+ intermediate monocytes and the total number of microvesicles **(E)**, and the percentage of CD54+ intermediate monocytes and the number of endothelium microvesicles **(F)** are shown.

subtypes in renal transplantation. The cells of the immune system communicate through cytokines and microparticles to maintain the homeostasis of the organism. Monocytes influence T-cell differentiation by antigenic presentation, release of cytokines, or cell-cell communications (103). The present results showed the correlations of different phenotypes of lymphocytes with the three different subsets of monocytes and the expression of CD86 and CD54. Despite the renal transplantation, the leading cause of CKD and the co-morbidities persist.

Consequently, the microinflammation process continues, based on the persistence of the main cause of the disease and the alteration of renal alteration function (showed by a decreased GFR compared to with HS), which can modulate the different subsets of leukocytes in patients who undergo transplantation. Despite the immunosuppressive treatment, the monocytes are influenced by these effects. This leads to further alteration of the vascular endothelium, resulting in adverse cardiovascular outcomes. This is more important in the interaction between cytotoxic T-cells and endothelial MVs, leading to an increased risk of atherogenic complications in patients with transplantation.

Even though the promising results of this work, the vast variety of treatment, not only immunosuppression, but also concomitant medications such as statins and allopurinol, that CKD patients suffers complicates the study and analysis of these patients. Most of this concomitant medication has anti-inflammatory effects (104–107) and affected immune phenotypes (108–111). Also, said medication can change the number and content of MVs (112–114).

The main limitation of this study is the number of volunteer HS of the same socioeconomic status (2), which is an important factor influencing the outcome of the disease. Furthermore, the wide variety of immunosuppressive treatment options, as well as concomitant meditation and comorbidities, complicate the study of the effects of the drugs in monocytes and MV subsets. However, this study provides original and integrative knowledge regarding the differences and relationships of leukocyte subpopulations. This could lead to a better comprehension of the participation of the immune function in negative outcomes in patients who undergo transplantation.

In conclusion, B-cell lymphopenia and an increase in the expression of costimulatory and adhesion molecules were observed in patients with KT. These changes were interrelated and associated with the number of MVs. These findings can partially explain the negatives outcomes of cardiovascular disease in patients with renal transplantations and the persistence of adverse renal outcomes. Further prospective studies are warranted to elucidate this communication mechanism and its role in negative outcomes. The increase in risk factor linked to CKD and the high cost associated with renal substitutive therapies could bring a heavy burden to public healthcare systems in the near future.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comité de ética de la investigación del Hospital Universitario 12 de Octubre. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

EM and JCarr conceived and designed the study. IG, CY, JCaro, and EM selected the patients and collected clinical and lymphocyte phenotype data. NC, GV, NS, CO, AF, and JCarr carried out the monocyte experiments. NC analyzed, collected monocyte data, carried out the graphical design, statistical analysis, and data interpretation. NS, AF, MA, RR, and JCarr performed the microvesicles experiments and analysis. NC and GV drafted the manuscript. MA, RR, EM, and JCarr edited and revised the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by the Instituto de Salud Carlos III through the project PI17/01029, PI19/00240, and PI20/01321 (cofounded by the European Regional Development Fund A way to make Europe), and Sociedad Española de Nefrologia. NC was a fellow of the program Contratos Asociados a Proyectos de Investigación, Instituto de Investigación Sanitaria Hospital 12 de Octubre (imas12), Madrid, Spain. NS was a fellow of the program Becas Asociadas a Proyectos de Investigación, Instituto de Investigación Sanitaria Hospital 12 de Octubre (imas12), Madrid, Spain. AF was a fellow of the program Contratos Predoctorales de Investigación en Salud, Instituto de Salud Carlos III (FI20/00018). GV received a granted from the Comunidad de Madrid y Fondo Social Europeo (PEJ-2020-AI/BMD-18141).

ACKNOWLEDGMENTS

This manuscript was edited for English Language by Charlesworth Author Services (www.cwauthors.com).

REFERENCES

1. Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B, et al. Chronic kidney disease: global dimension and perspectives. *Lancet.* (2013) 382:260–72. doi: 10.1016/S0140-6736(13)60687-X
2. Bikbov B, Purcell CA, Levey AS, Smith M, Abdoli A, Abebe M, et al. Global, regional, and national burden of chronic kidney disease, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet.* (2020) 395:709–33. doi: 10.1016/S0140-6736(20)30045-3
3. Ortiz A, Sanchez-Niño MD, Crespo-Barrio M, De-Sequera-Ortiz P, Fernández-Giráldez E, García-Maset R, et al. The Spanish Society of Nephrology (SENEFRO) commentary to the Spain GBD 2016 report: keeping chronic kidney disease out of sight of health authorities will only magnify the problem. *Nefrologia.* (2019) 39:29–34. doi: 10.1016/j.nefro.2018.09.002
4. Carracedo J, Alique M, Vida C, Bodega G, Ceprián N, Morales E, et al. Mechanisms of cardiovascular disorders in patients with chronic kidney disease: a process related to accelerated senescence. *Front Cell Dev Biol.* (2020) 8:185. doi: 10.3389/fcell.2020.00185
5. Xie Y, Bowe B, Mokdad AH, Xian H, Yan Y, Li T, et al. Analysis of the Global Burden of Disease study highlights the global, regional, and national trends of chronic kidney disease epidemiology from 1990 to 2016. *Kidney Int.* (2018) 94:567–81. doi: 10.1016/j.kint.2018.04.011
6. Levey AS, Coresh J. Chronic kidney disease. *Lancet.* (2012) 379:165–80. doi: 10.1016/S0140-6736(11)60178-5
7. Glorieux G, Cohen G, Jankowski J, Vanholder R. Platelet/leukocyte activation, inflammation, and uremia. *Semin Dial.* (2009) 22:423–7. doi: 10.1111/j.1525-139X.2009.00593.x
8. Mutsaers HAM, Stribos EGD, Glorieux G, Vanholder R, Olinga P. Chronic kidney disease and fibrosis: the role of uremic retention solutes. *Front Med.* (2015) 2:60. doi: 10.3389/fmed.2015.00060
9. Claro LM, Moreno-Amaral AN, Gadotti AC, Dolenga CJ, Nakao LS, Azevedo MLV, et al. The impact of uremic toxicity induced inflammatory response on the cardiovascular burden in chronic kidney disease. *Toxins (Basel).* (2018) 10:384. doi: 10.3390/toxins10100384
10. Kaminski TW, Pawlak K, Karbowska M, Mysliwiec M, Grzegorzewski W, Kuna J, et al. Association between uremic toxin-anthranilic acid and fibrinolytic system activity in predialysis patients at different stages of chronic kidney disease. *Int Urol Nephrol.* (2018) 50:127–35. doi: 10.1007/s11255-017-1729-1
11. Kooman JP, Dekker MJ, Usvyat LA, Kotanko P, van der Sande FM, Schalkwijk CG, et al. Inflammation and premature aging in advanced chronic kidney disease. *Am J Physiol Renal Physiol.* (2017) 313:F938–F50. doi: 10.1152/ajprenal.00256.2017
12. Inker LA, Astor BC, Fox CH, Isakova T, Lash JP, Peralta CA, et al. KDOQI US commentary on the 2012 KDIGO clinical practice guideline for the evaluation and management of CKD. *Am J Kidney Dis.* (2014) 63:713–35. doi: 10.1053/j.ajkd.2014.01.416
13. Chapter 1: Definition classification of CKD. *Kidney Int Suppl (2011).* (2013) 3:19–62. doi: 10.1038/kisup.2012.64
14. Chapter 3: Management of progression complications of CKD. *Kidney Int Suppl 2011.* (2013) 3:73–90. doi: 10.1038/kisup.2012.66
15. Alicic RZ, Johnson EJ, Tuttle KR. Inflammatory mechanisms as new biomarkers and therapeutic targets for diabetic kidney disease. *Adv Chronic Kidney Dis.* (2018) 25:181–91. doi: 10.1053/j.ackd.2017.12.002
16. Silverstein DM. Inflammation in chronic kidney disease: role in the progression of renal and cardiovascular disease. *Pediatr Nephrol.* (2009) 24:1445–52. doi: 10.1007/s00467-008-1046-0
17. Ebert T, Pawelzik SC, Witasp A, Arefin S, Hobson S, Kublickiene K, et al. Inflammation and premature ageing in chronic kidney disease. *Toxins (Basel).* (2020) 12:227. doi: 10.3390/toxins12040227
18. Crépin T, Legendre M, Carron C, Vachey C, Courivaud C, Rebibou JM, et al. Uraemia-induced immune senescence and clinical outcomes in chronic kidney disease patients. *Nephrol Dial Transplant.* (2020) 35:624–32. doi: 10.1093/ndt/gfy276
19. Franceschi C, Bonafè M, Valensin S, Olivieri F, De Luca M, Ottaviani E, et al. Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann N Y Acad Sci.* (2000) 908:244–54. doi: 10.1111/j.1749-6632.2000.tb06651.x
20. Fuente M, Miquel J. An update of the oxidation-inflammation theory of aging: the involvement of the immune system in oxi-inflamm-aging. *Curr Pharm Des.* (2009) 15:3003–26. doi: 10.2174/138161209789058110
21. Syed-Ahmed M, Narayanan M. Immune dysfunction and risk of infection in chronic kidney disease. *Adv Chronic Kidney Dis.* (2019) 26:8–15. doi: 10.1053/j.ackd.2019.01.004
22. Kato S, Chmielewski M, Honda H, Pecoits-Filho R, Matsuo S, Yuzawa Y, et al. Aspects of immune dysfunction in end-stage renal disease. *Clin J Am Soc Nephrol.* (2008) 3:1526–33. doi: 10.2215/CJN.00950208
23. Kurts C, Panzer U, Anders HJ, Rees AJ. The immune system and kidney disease: basic concepts and clinical implications. *Nat Rev Immunol.* (2013) 13:738–53. doi: 10.1038/nri3523

24. Mihai S, Codrici E, Popescu ID, Enciu AM, Albulescu L, Necula LG, et al. Inflammation-related mechanisms in chronic kidney disease prediction, progression, and outcome. *J Immunol Res.* (2018) 2018:2180373. doi: 10.1155/2018/2180373
25. Bottazzi B, Riboli E, Mantovani A. Aging, inflammation and cancer. *Semin Immunol.* (2018) 40:74–82. doi: 10.1016/j.smim.2018.10.011
26. Jiménez R, Ramírez R, Carracedo J, Agüera M, Navarro D, Santamaría R, et al. Cytometric bead array (CBA) for the measurement of cytokines in urine and plasma of patients undergoing renal rejection. *Cytokine.* (2005) 32:45–50. doi: 10.1016/j.cyto.2005.07.009
27. Merino A, Buendia P, Martin-Malo A, Aljama P, Ramirez R, Carracedo J. Senescent CD14+CD16+ monocytes exhibit proinflammatory and proatherosclerotic activity. *J Immunol.* (2011) 186:1809–15. doi: 10.4049/jimmunol.1001866
28. Sato Y, Yanagita M. Immunology of the ageing kidney. *Nat Rev Nephrol.* (2019) 15:625–40. doi: 10.1038/s41581-019-0185-9
29. Carracedo J, Buendía P, Merino A, Soriano S, Esquivias E, Martín-Malo A, et al. Cellular senescence determines endothelial cell damage induced by uremia. *Exp Gerontol.* (2013) 48:766–73. doi: 10.1016/j.exger.2013.04.004
30. Bernelot Moens SJ, Verweij SL, van der Valk FM, van Capelleveen JC, Kroon J, Versloot M, et al. Arterial and cellular inflammation in patients with CKD. *J Am Soc Nephrol.* (2017) 28:1278–85. doi: 10.1681/ASN.2016030317
31. Roberts MA, Hare DL, Ratnaike S, Ierino FL. Cardiovascular biomarkers in CKD: pathophysiology and implications for clinical management of cardiac disease. *Am J Kidney Dis.* (2006) 48:341–60. doi: 10.1053/j.ajkd.2006.06.005
32. Heine GH, Ortiz A, Massy ZA, Lindholm B, Wiecek A, Martínez-Castelao A, et al. Monocyte subpopulations and cardiovascular risk in chronic kidney disease. *Nat Rev Nephrol.* (2012) 8:362–9. doi: 10.1038/nrneph.2012.41
33. Rogacev KS, Seiler S, Zawada AM, Reichart B, Herath E, Roth D, et al. CD14++CD16+ monocytes and cardiovascular outcome in patients with chronic kidney disease. *Eur Heart J.* (2011) 32:84–92. doi: 10.1093/eurheartj/ehq371
34. Alique M, Ruíz-Torres MP, Bodega G, Noci MV, Troyano N, Bohórquez L, et al. Microvesicles from the plasma of elderly subjects and from senescent endothelial cells promote vascular calcification. *Aging (Albany NY).* (2017) 9:778–89. doi: 10.18632/aging.101191
35. Camussi G, Deregibus MC, Bruno S, Cantaluppi V, Biancone L. Exosomes/microvesicles as a mechanism of cell-to-cell communication. *Kidney Int.* (2010) 78:838–48. doi: 10.1038/ki.2010.278
36. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. *J Cell Biol.* (2013) 200:373–83. doi: 10.1083/jcb.201211138
37. Ståhl A, Johansson K, Mossberg M, Kahn R, Karpman D. Exosomes and microvesicles in normal physiology, pathophysiology, and renal diseases. *Pediatr Nephrol.* (2019) 34:11–30. doi: 10.1007/s00467-017-3816-z
38. Buendía P, De Oca AM, Madueño JA, Merino A, Martín-Malo A, Aljama P, et al. Endothelial microparticles mediate inflammation-induced vascular calcification. *FASEB J.* (2015) 29:173–81. doi: 10.1096/fj.14-249706
39. Garcia GG, Harden P, Chapman J. The global role of kidney transplantation. *Kidney Blood Press Res.* (2012) 35:299–304. doi: 10.1159/000337044
40. Kostro JZ, Hellmann A, Kobiela J, Skóra I, Lichodziejewska-Niemierko M, Debska-lizień A, et al. Quality of life after kidney transplantation: a prospective study. *Transplant Proc.* (2016) 48:50–4. doi: 10.1016/j.transproceed.2015.10.058
41. Ozcan H, Yucel A, Avşar UZ, Cankaya E, Yucel N, Gözübüyük H, et al. Kidney transplantation is superior to hemodialysis and peritoneal dialysis in terms of cognitive function, anxiety, and depression symptoms in chronic kidney disease. *Transplant Proc.* (2015) 47:1348–51. doi: 10.1016/j.transproceed.2015.04.032
42. Legendre C, Canaud G, Martinez F. Factors influencing long-term outcome after kidney transplantation. *Transpl Int.* (2014) 27:19–27. doi: 10.1111/tri.12217
43. Mix TCH, Kazmi W, Khan S, Ruthazer R, Rohrer R, Pereira BJG, et al. Anemia: a continuing problem following kidney transplantation. *Am J Transplant.* (2003) 3:1426–33. doi: 10.1046/j.1600-6135.2003.00224.x
44. Aveles PR, Criminácio CR, Gonçalves S, Bignelli AT, Claro LM, Siqueira SS, et al. Association between biomarkers of carbonyl stress with increased systemic inflammatory response in different stages of chronic kidney disease and after renal transplantation. *Nephron Clin Pract.* (2010) 116:c294–c9. doi: 10.1159/000318792
45. Kasiske BL, Maclean JR, Snyder JJ. Acute myocardial infarction and kidney transplantation. *J Am Soc Nephrol.* (2006) 17:900–7. doi: 10.1681/ASN.2005090984
46. Recio-Mayoral A, Banerjee D, Streather C, Kaski JC. Endothelial dysfunction, inflammation and atherosclerosis in chronic kidney disease - a cross-sectional study of predialysis, dialysis and kidney-transplantation patients. *Atherosclerosis.* (2011) 216:446–51. doi: 10.1016/j.atherosclerosis.2011.02.017
47. Pérez-Sáez MJ, Herrera S, Prieto-Alhambra D, Vilaplana L, Nogués X, Vera M, et al. Bone density, microarchitecture, and material strength in chronic kidney disease patients at the time of kidney transplantation. *Osteoporos Int.* (2017) 28:2723–7. doi: 10.1007/s00198-017-4065-5
48. Molina M, Allende LM, Ramos LE, Gutiérrez E, Pleguezuelo DE, Hernández ER, et al. CD19+ B-cells, a new biomarker of mortality in hemodialysis patients. *Front Immunol.* (2018) 9:1221. doi: 10.3389/fimmu.2018.01221
49. Carmona A, Agüera ML, Luna-Ruiz C, Buendía P, Calleros L, García-Jerez A, et al. Markers of endothelial damage in patients with chronic kidney disease on hemodialysis. *Am J Physiol Renal Physiol.* (2017) 312:F673–F81. doi: 10.1152/ajprenal.00013.2016
50. Merino A, Portolés J, Selgas R, Ojeda R, Buendia P, Ocaña J, et al. Effect of different dialysis modalities on microinflammatory status and endothelial damage. *Clin J Am Soc Nephrol.* (2010) 5:227–34. doi: 10.2215/CJN.03260509
51. Lim MA, Kohli J, Bloom RD. Immunosuppression for kidney transplantation: where are we now and where are we going? *Transplant Rev.* (2017) 31:10–7. doi: 10.1016/j.trre.2016.10.006
52. de Mattos AM, Olyaei AJ, Bennett WM. Nephrotoxicity of immunosuppressive drugs: long-term consequences and challenges for the future. *Am J Kidney Dis.* (2000) 35:333–46. doi: 10.1016/S0272-6386(00)70348-9
53. Karuthu S, Blumberg EA. Common infections in kidney transplant recipients. *Clin J Am Soc Nephrol.* (2012) 7:2058–70. doi: 10.2215/CJN.04410512
54. Au E, Wong G, Chapman JR. Cancer in kidney transplant recipients. *Nat Rev Nephrol.* (2018) 14:508–20. doi: 10.1038/s41581-018-0022-6
55. Muntean A, Lucan M. Immunosuppression in kidney transplantation. *Clujul Med.* (2013) 86:177–80.
56. Hutchinson JA, Geissler EK. Now or never? The case for cell-based immunosuppression in kidney transplantation. *Kidney Int.* (2015) 87:1116–24. doi: 10.1038/ki.2015.50
57. Marcen R. Immunosuppressive drugs in kidney transplantation: Impact on patient survival, and incidence of cardiovascular disease, malignancy and infection. *Drugs.* (2009) 69:2227–43. doi: 10.2165/11319260-000000000-00000
58. Granata S, Dalla Gassa A, Bellin G, Lupo A, Zaza G. Transcriptomics: a step behind the comprehension of the polygenic influence on oxidative stress, immune deregulation, and mitochondrial dysfunction in chronic kidney disease. *Biomed Res Int.* (2016) 2016:9290857. doi: 10.1155/2016/9290857
59. Petrányi GG. The complexity of immune and alloimmune response. *Transpl Immunol.* (2002) 10:91–100. doi: 10.1016/S0966-3274(02)00054-0
60. Trivedi HL. Immunobiology of rejection and adaptation. *Transplant Proc.* (2007) 39:647–52. doi: 10.1016/j.transproceed.2007.01.047
61. Zaza G, Pontrelli P, Pertosa G, Granata S, Rossini M, Porreca S, et al. Dialysis-related systemic microinflammation is associated with specific genomic patterns. *Nephrol Dial Transplant.* (2008) 23:1673–81. doi: 10.1093/ndt/gfm804
62. Zaza G, Leventhal J, Signorini L, Gambaro G, Cravedi P. Effects of antirejection drugs on innate immune cells after kidney transplantation. *Front Immunol.* (2019) 10:2978. doi: 10.3389/fimmu.2019.02978
63. Klaus G, Mostert K, Reckzeh B, Mueller TF. Phenotypic changes in lymphocyte subpopulations in pediatric renal-transplant patients after T-cell depletion. *Transplantation.* (2003) 76:1719–24. doi: 10.1097/01.TP.0000100396.81490.0C
64. Moreso F, Torres IB, Martínez-Gallo M, Benlloch S, Cantarell C, Perelló M, et al. Gene expression signature of tolerance and lymphocyte subsets in stable renal transplants: results of a cross-sectional study. *Transpl Immunol.* (2014) 31:11–6. doi: 10.1016/j.trim.2014.04.008

65. Fernández-Fresnedo G, Ramos MA, González-Pardo MC, De Francisco ALM, López-Hoyos M, Arias M. B lymphopenia in uraemia is related to an accelerated *in vitro* apoptosis and dysregulation of Bcl-2. *Nephrol Dial Transplant*. (2000) 15:502–10. doi: 10.1093/ndt/15.4.502
66. Xiang FF, Zhu JM, Cao XS, Shen B, Zou JZ, Liu ZH, et al. Lymphocyte depletion and subset alteration correlate to renal function in chronic kidney disease patients. *Ren Fail.* (2016) 38:7–14. doi: 10.3109/0886022X.2015.1106871
67. Kho MML, Bouvy AP, Cadogan M, Kraaijeveld R, Baan CC, Weimar W. The effect of low and ultra-low dosages Thymoglobulin on peripheral T, B and NK cells in kidney transplant recipients. *Transpl Immunol.* (2012) 26:186–90. doi: 10.1016/j.trim.2012.02.003
68. Ducloux D, Challier B, Saas P, Tiberghien P, Chalopin JM. CD4 cell lymphopenia and atherosclerosis in renal transplant recipients. *J Am Soc Nephrol.* (2003) 14:767–72. doi: 10.1097/01.ASN.0000048718.43419.44
69. Ducloux D, Courivaud C, Bamoulid J, Vivet B, Chabroux A, Deschamps M, et al. Prolonged CD4 T cell lymphopenia increases morbidity and mortality after renal transplantation. *J Am Soc Nephrol.* (2010) 21:868–75. doi: 10.1681/ASN.2009090976
70. Ramírez R, Carracedo J, Merino A, Soriano S, Rodríguez R, Blasco M, et al. Stress-induced premature senescence in mononuclear cells from patients on long-term hemodialysis. *Am J Kidney Dis.* (2005) 45:353–9. doi: 10.1053/j.ajkd.2004.10.022
71. Ramírez R, Carracedo J, Merino A, Soriano S, Ojeda R, Alvarez-Lara MA, et al. CD14+CD16+ monocytes from chronic kidney disease patients exhibit increased adhesion ability to endothelial cells. *Contrib Nephrol.* (2011) 171:57–61. doi: 10.1159/000327134
72. Fingerle-Rowson G, Angstwurm M, Andreesen R, Ziegler-Heitbrock HWL. Selective depletion of CD14+ CD16+ monocytes by glucocorticoid therapy. *Clin Exp Immunol.* (1998) 112:501–6. doi: 10.1046/j.1365-2249.1998.00617.x
73. Dayyani F, Belge K, Frankenberger M, Mack M, Berki T, Ziegler-Heitbrock L. Mechanism of glucocorticoid-induced depletion of human CD14+CD16+ monocytes. *J Leukoc Biol.* (2003) 74:33–9. doi: 10.1189/jlb.1202612
74. Rogacev KS, Zawada AM, Hundsdorfer J, Achenbach M, Held G, Fliser D, et al. Immunosuppression and monocyte subsets. *Nephrol Dial Transplant.* (2015) 30:143–53. doi: 10.1093/ndt/gfu315
75. Girndt M, Sester M, Sester U, Kaul H, Köhler H. Defective expression of B7-2 (CD86) on monocytes of dialysis patients correlates to the uremia-associated immune defect. *Kidney Int.* (2001) 59:1382–9. doi: 10.1046/j.1523-1755.2001.0590041382.x
76. Verkade MA, van Druningen CJ, Vaessen LMB, Hesselink DA, Weimar W, Betjes MGH. Functional impairment of monocyte-derived dendritic cells in patients with severe chronic kidney disease. *Nephrol Dial Transplant.* (2007) 22:128–38. doi: 10.1093/ndt/gfl519
77. Dopheide JF, Sester U, Schlitt A, Horstick G, Rupprecht HJ, Münzel T, et al. Monocyte-derived dendritic cells of patients with coronary artery disease show an increased expression of costimulatory molecules CD40, CD80 and CD86 *in vitro*. *Coron Artery Dis.* (2007) 18:523–31. doi: 10.1097/MCA.0b013e3282eff1ad
78. Presicce P, Giannelli S, Taddeo A, Villa ML, Della Bella S. Human defensins activate monocyte-derived dendritic cells, promote the production of proinflammatory cytokines, and up-regulate the surface expression of CD91. *J Leukoc Biol.* (2009) 86:941–8. doi: 10.1189/jlb.0708412
79. Vincenti F. Costimulation blockade in autoimmunity and transplantation. *J Allergy Clin Immunol.* (2008) 121:299–306. doi: 10.1016/j.jaci.2008.01.002
80. Poirier N, Blancho G, Vanhove B. A more selective costimulatory blockade of the CD28-B7 pathway. *Transpl Int.* (2011) 24:2–11. doi: 10.1111/j.1432-2277.2010.01176.x
81. Jonker M, Ossevoort MA, Vierboom M. Blocking the CD80 and CD86 costimulation molecules: lessons to be learned from animal models. *Transplantation.* (2002) 73(1 Suppl.):S23–S6. doi: 10.1097/00007890-200201151-00009
82. Bhatia S, Edidin M, Almo SC, Nathenson SG. B7-1 and B7-2: similar costimulatory ligands with different biochemical, oligomeric and signaling properties. *Immunol Lett.* (2006) 104:70–5. doi: 10.1016/j.imlet.2005.11.019
83. Beyersdorf N, Kerkau T, Hünig T. CD28 co-stimulation in T-cell homeostasis: a recent perspective. *Immunotargets Ther.* (2015) 4:111–22. doi: 10.2147/ITT.S61647
84. Bishop GA, Hall BM. Expression of leucocyte and lymphocyte adhesion molecules in the human kidney. *Kidney Int.* (1989) 36:1078–85. doi: 10.1038/ki.1989.303
85. Schwarz C, Regele H, Steininger R, Hansmann C, Mayer G, Oberbauer R. The contribution of adhesion molecule expression in donor kidney biopsies to early allograft dysfunction. *Transplantation.* (2001) 71:1666–70. doi: 10.1097/00007890-200106150-00028
86. Glomsda BA, Blaheta RA, Hailer NP. Inhibition of monocyte/endothelial cell interactions and monocyte adhesion molecule expression by the immunosuppressant mycophenolate mofetil. *Spinal Cord.* (2003) 41:610–9. doi: 10.1038/sj.sc.3101512
87. Wong KL, Yeap WH, Tai JJY, Ong SM, Dang TM, Wong SC. The three human monocyte subsets: implications for health and disease. *Immunol Res.* (2012) 53:41–57. doi: 10.1007/s12026-012-8297-3
88. Skrzeczyńska-Moncznik J, Bzowska M, Loseke S, Grage-Griebenow E, Zembala M, Pryjma J. Peripheral blood CD14high CD16+ monocytes are main producers of IL-10. *Scand J Immunol.* (2008) 67:152–9. doi: 10.1111/j.1365-3083.2007.02051.x
89. Briggs JD. Causes of death after renal transplantation. *Nephrol Dial Transplant.* (2001) 16:1545–9. doi: 10.1093/ndt/16.8.1545
90. Wong CK, Lit LC, Tam LS, Li EK, Lam CW. Aberrant production of soluble costimulatory molecules CTLA-4, CD28, CD80 and CD86 in patients with systemic lupus erythematosus. *Rheumatology (Oxford).* (2005) 44:989–94. doi: 10.1093/rheumatology/keh663
91. Uccioli L, Sinistro A, Almerighi C, Ciaprini C, Cavazza A, Giurato L, et al. Proinflammatory modulation of the surface and cytokine phenotype of monocytes in patients with acute Charcot foot. *Diabetes Care.* (2010) 33:350–5. doi: 10.2337/dc09-1141
92. Cinova J, Palová-Jelínková L, Smythies LE, Cerná M, Pecharová B, Dvorák M, et al. Gliadin peptides activate blood monocytes from patients with celiac disease. *J Clin Immunol.* (2007) 27:201–9. doi: 10.1007/s10875-006-9061-z
93. Ohno SI, Ishikawa A, Kuroda M. Roles of exosomes and microvesicles in disease pathogenesis. *Adv Drug Deliv Rev.* (2013) 65:398–401. doi: 10.1016/j.addr.2012.07.019
94. Inal JM, Ansa-Addo EA, Stratton D, Kholia S, Antwi-Baffour SS, Jorfi S, et al. Microvesicles in health and disease. *Arch Immunol Ther Exp (Warsz).* (2012) 60:107–21. doi: 10.1007/s00005-012-0165-2
95. Chen Y, Li G, Liu ML. Microvesicles as emerging biomarkers and therapeutic targets in cardiometabolic diseases. *Genomics Proteomics Bioinformatics.* (2018) 16:50–62. doi: 10.1016/j.gpb.2017.03.006
96. Zaldivia MTK, McFadyen JD, Lim B, Wang X, Peter K. Platelet-derived microvesicles in cardiovascular diseases. *Front Cardiovasc Med.* (2017) 4:74. doi: 10.3389/fcvm.2017.00074
97. Badimon L, Suades R, Arderiu G, Peña E, Chiva-Blanch G, Padró T. Microvesicles in atherosclerosis and angiogenesis: from bench to bedside and reverse. *Front Cardiovasc Med.* (2017) 4:77. doi: 10.3389/fcvm.2017.00077
98. Alique M, Bodega G, Corchete E, García-Menéndez E, de Sequera P, Luque R, et al. Microvesicles from indoxyl sulfate-treated endothelial cells induce vascular calcification *in vitro*. *Comput Struct Biotechnol J.* (2020) 18:953–66. doi: 10.1016/j.csbj.2020.04.006
99. Carmona A, Guerrero F, Buendia P, Obrero T, Aljama P, Carracedo J. Microvesicles derived from indoxyl sulfate treated endothelial cells induce endothelial progenitor cells dysfunction. *Front Physiol.* (2017) 8:666. doi: 10.3389/fphys.2017.00666
100. Thaler J, Ay C, Mackman N, Bertina RM, Kaider A, Marosi C, et al. Microparticle-associated tissue factor activity, venous thromboembolism and mortality in pancreatic, gastric, colorectal and brain cancer patients. *J Thromb Haemost.* (2012) 10:1363–70. doi: 10.1111/j.1538-7836.2012.04754.x
101. Suades R, Padró T, Alonso R, Mata P, Badimon L. High levels of TSP1+/CD142+ platelet-derived microparticles characterise young patients with high cardiovascular risk and subclinical atherosclerosis. *Thromb Haemost.* (2015) 114:1310–21. doi: 10.1160/TH15-04-0325
102. Berezin A. Pattern of micro vesicles in heart failure: novel biomarker of endothelial dysfunction and vascular reparation. *Biomarkers J.* (2018) 4:16. doi: 10.21767/2472-1646.100054
103. Geissmann F, Auffray C, Palframan R, Wirrig C, Ciocca A, Campisi L, et al. Blood monocytes: distinct subsets, how they relate to dendritic cells, and

their possible roles in the regulation of T-cell responses. *Immunol Cell Biol.* (2008) 86:398–408. doi: 10.1038/icb.2008.19
104. Bucharles S, Barberato SH, Stinghen AEM, Gruber B, Piekala L, Dambiski AC, et al. Impact of cholecalciferol treatment on biomarkers of inflammation and myocardial structure in hemodialysis patients without hyperparathyroidism. *J Ren Nutr.* (2012) 22:284–91. doi: 10.1053/j.jrn.2011.07.001
105. Gamboa JL, Pretorius M, Todd-Tzanetos DR, Luther JM, Yu C, Ikizler TA, et al. Comparative effects of angiotensin-converting enzyme inhibition and angiotensin-receptor blockade on inflammation during hemodialysis. *J Am Soc Nephrol.* (2012) 23:334–42. doi: 10.1681/ASN.2011030287
106. Heimbürger O, Stenvinkel P. Statins to treat chronic inflammation in dialysis patients–is this feasible? *Perit Dial Int.* (2007) 27:254–7. doi: 10.1177/089686080702700308
107. Vlachopoulos C, Aznaouridis K, Dagre A, Vasiliadou C, Masoura C, Stefanadi E, et al. Protective effect of atorvastatin on acute systemic inflammation-induced endothelial dysfunction in hypercholesterolaemic subjects. *Eur Heart J.* (2007) 28:2102–9. doi: 10.1093/eurheartj/ehm247
108. Rodríguez-Perea AL, Montoya CJ, Olek S, Chougnet CA, Velilla PA. Statins increase the frequency of circulating CD4+ FOXP3+ regulatory T cells in healthy individuals. *J Immunol Res.* (2015) 2015:762506. doi: 10.1155/2015/762506
109. Yadav A, Betts MR, Collman RG. Statin modulation of monocyte phenotype and function: implications for HIV-1-associated neurocognitive disorders. *J Neurovirol.* (2016) 22:584–96. doi: 10.1007/s13365-016-0433-8
110. Rothe G, Herr AS, Stöhr J, Abletshauser C, Weidinger G, Schmitz G. A more mature phenotype of blood mononuclear phagocytes is induced by fluvastatin treatment in hypercholesterolemic patients with coronary heart disease. *Atherosclerosis.* (1999) 144:251–61. doi: 10.1016/S0021-9150(99)00061-1
111. Fisher SA, Rahimzadeh M, Brierley C, Gration B, Doree C, Kimber CE, et al. The role of vitamin D in increasing circulating T regulatory cell numbers and modulating T regulatory cell phenotypes in patients with inflammatory disease or in healthy volunteers: a systematic review. *PLoS One.* (2019) 14:e0222313. doi: 10.1371/journal.pone.0222313
112. Chiva-Blanch G, Badimon L. Cross-talk between lipoproteins and inflammation: the role of microvesicles. *J Clin Med.* (2019) 8:2059. doi: 10.3390/jcm8122059
113. Badimon L, Suades R, Fuentes E, Palomo I, Padró T. Role of platelet-derived microvesicles as crosstalk mediators in atherothrombosis and future pharmacology targets: a link between inflammation, atherosclerosis, and thrombosis. *Front Pharmacol.* (2016) 7:293. doi: 10.3389/fphar.2016.00293
114. Wallace A, Albadawi H, Hoang P, Fleck A, Naidu S, Knuttinen G, et al. Statins as a preventative therapy for venous thromboembolism. *Cardiovasc Diagn Ther.* (2017) 7(Suppl 3):S207–S18. doi: 10.21037/cdt.2017.09.12

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

ORIGINAL RESEARCH
published: 20 October 2021
doi: 10.3389/fmed.2021.724316

The Association of Circulating CD14++CD16+ Monocytes, Natural Killer Cells and Regulatory T Cells Subpopulations With Phenotypes of Cardiovascular Disease in a Cohort of Peritoneal Dialysis Patients

*Anila Duni[1], Georgios Vartholomatos[2], Olga Balafa[1], Margarita Ikonomou[1], Paraskevi Tseke[3], Lampros Lakkas[4], Karolos Paulos Rapsomanikis[1], Athanasios Kitsos[1], Ioanna Theodorou[1], Charalambos Pappas[1], Katerina K. Naka[4], Michael Mitsis[5,6] and Evangelia Dounousi[1,7]**

[1] Department of Nephrology, University Hospital of Ioannina, Ioannina, Greece, [2] Laboratory of Haematology - Unit of Molecular Biology, University Hospital of Ioannina, Ioannina, Greece, [3] Renal Unit, General Hospital Alexandra, Athens, Greece, [4] Second Department of Cardiology and Michaelidion Cardiac Center, Medical School University of Ioannina, Ioannina, Greece, [5] Department of Surgery, University Hospital of Ioannina, Ioannina, Greece, [6] Department of Surgery, School of Medicine, University of Ioannina, Ioannina, Greece, [7] Department of Nephrology, School of Medicine, University of Ioannina, Ioannina, Greece

The altered expression of immune cells including monocyte subsets, natural killer (NK) cells and CD4+CD25+ regulatory T cells (Tregs) in end-stage kidney disease, affect the modulation of inflammation and immunity with significant clinical implications. The aim of this study was to investigate the profile of specific immune cells subpopulations and their correlations with phenotypes of established cardiovascular disease (CVD), including coronary artery disease (CAD) and heart failure (HF) in peritoneal dialysis (PD) patients.

Materials and Methods: 29 stable PD patients and 13 healthy volunteers were enrolled. Demographic, laboratory, bioimpedance measurements, lung ultrasound and echocardiography data were collected. The peripheral blood immune cell subsets analysis was performed using flow cytometry.

Results: PD patients compared to normal controls had lower total lymphocytes (22.3 ± 6.28 vs. 31.3 ± 5.54%, $p = <0.001$) and B-lymphocytes (6.39 ± 3.75 vs. 9.72 ± 3.63%, $p = 0.01$) as well as higher CD14++CD16+ monocytes numbers (9.28 ± 6.36 vs. 4.75 ± 2.75%, $p = 0.0002$). PD patients with prevalent CAD had NK cells levels elevated above median values (85.7 vs. 40.9%, $p = 0.04$) and lower B cells counts (3.85 ± 2.46 vs. 7.2 ± 3.77%, $p = 0.03$). Patients with increased NK cells (>15.4%) had 3.8 times higher risk of CAD comparing with patients with lower NK cell levels (95% CI, 1.86 – 77.87; $p = 0.034$). B cells were inversely associated with the presence of CAD (increase of B-lymphocyte by 1% was associated with 30% less risk for presence of CAD (95% CI, −0.71 – 0.01; $p = 0.05$). Overhydrated patients had lower lymphocytes counts (18.3 ± 4.29% vs. 24.7 ± 6.18%, $p = 0.006$) and increased NK cells [20.5% (14.3, 23.6)

OPEN ACCESS

Edited by:
Maria J. Stangou, Aristotle University of Thessaloniki, Greece

Reviewed by:
Katarzyna Laura Kotfis, Pomeranian Medical University, Poland
Konstantinos Bantis, Hippokration General Hospital of Thessaloniki, Greece

***Correspondence:**
Evangelia Dounousi
evangeldou@gmail.com

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: 12 June 2021
Accepted: 20 September 2021
Published: 20 October 2021

Citation:
Duni A, Vartholomatos G, Balafa O, Ikonomou M, Tseke P, Lakkas L, Rapsomanikis KP, Kitsos A, Theodorou I, Pappas C, Naka KK, Mitsis M and Dounousi E (2021) The Association of Circulating CD14++CD16+ Monocytes, Natural Killer Cells and Regulatory T Cells Subpopulations With Phenotypes of Cardiovascular Disease in a Cohort of Peritoneal Dialysis Patients. Front. Med. 8:724316. doi: 10.3389/fmed.2021.724316

vs. 13.21% (6.23, 19.2), $p = 0.04$)]. In multiple logistic regression analysis the CRP (OR 1.43; 95% CI, 1.00 – 2.05; $p = 0.04$)] and lymphocytes counts (OR 0.79; 95% CI, 0.63–0.99; $p = 0.04$)] were associated with the presence of lung comets. Patients with higher NK cells (>15.4%, $n = 15$) were more likely to be rapid transporters (D/P creatinine 0.76 ± 0.1 vs. 0.69 ± 0.08, $p = 0.04$). Patients displaying higher Tregs (>1.79%) were older (70.8 ± 10.7 years vs. 57.7 ± 14.7years, $p = 0.011$) and had higher nPCR (0.83 ± 0.14 vs. 0.91 ± 0.17, $p = 0.09$).

Conclusion: Future research is required to evaluate the role of immune cells subsets as potential tools to identify patients at the highest risk for complications and guide interventions.

Keywords: CD14++CD16+ monocytes, natural killer cells, CD4+CD25+ regulatory T cells, coronary artery disease, overhydration, fast transporters

INTRODUCTION

The chronic inflammatory state is considered a hallmark of end-stage kidney disease (ESKD) and is considered to play a pivotal part in the pathogenesis and progression of the compound phenotypes of cardiovascular disease in chronic kidney disease (CKD), including accelerated atherosclerosis, left ventricular hypertrophy (LVH) and heart failure (1).

The complex derangement of the innate and acquired arms of the immune system in patients with CKD includes a vast array of pathogenic mechanisms and effectors. It has been suggested that the altered expression of the immune cells including monocyte subsets, natural killer (NK) cells as well as CD4+CD25+ regulatory T cells (Tregs) affects the modulation of inflammation and immunity with significant clinical implications (2). The three phenotypically and functionally distinct human monocyte subsets are specified by the expression of CD14 and CD16 surface antigens and include CD14++CD16– (classical), CD14++CD16+ (intermediate) and CD14+CD16++ (non-classical) monocytes (3). The pro-inflammatory CD14++CD16+ intermediate monocytes are characterized by upregulated chemokine receptors relevant to atherosclerosis, a high capacity for oxidized low-density lipoprotein (LDL) uptake as well as proangiogenic properties (3, 4). NK cells, apart from being essential players in innate immunity pathways, are currently considered to perform important functions that bridge the innate and acquired arms of the immune system, thus arranging adaptive immune responses and immunoregulation (5). Although their direct role in atherogenesis has been delineated, data regarding their role in heart failure are limited (6). CD4+CD25+FOXP3+ regulatory T cells (Tregs) are a specific subpopulation of T cells, comprising 5–10% of all peripheral CD4+ T cells. They hold a key position in the regulation of the intertwining pathways of immune homeostasis and tolerance with available evidence indicating a potential protective role against cardiovascular disease (7).

The pathophysiology of the chronic inflammatory state of ESKD in Peritoneal Dialysis (PD) patients includes various potential culprits such as the gradual loss of residual renal function, fluid overload, the endotoxinemia burden, imbalance of adipokines as well as the biocompatibility of the peritoneal dialysis solutions utilized (8, 9). Classical markers of inflammation such as C-reactive protein (CRP) and interleukin-6 levels are frequently increased in PD patients which in turn adversely affects cardiovascular risk as well as technique and patient survival (10, 11). The evaluation and validation of various biomarkers in PD as potential tools for improving patient management is currently a subject of extensive research (12). Accordingly, the associations of immune cell subpopulations as potential markers of inflammations with specific modality related as well as clinical outcomes remain to be determined in PD patients.

Thus, we conducted a pilot study in a cohort of PD patients so as to investigate the profile of specific subpopulations of immune cells in the circulation and their potential correlations with phenotypes of established cardiovascular disease (CVD), including coronary artery disease (CAD) and heart failure (HF), as well as related clinical and laboratory indices. In addition, associations of immune cells with the peritoneal membrane characteristic, dialysis adequacy and various inflammatory and nutritional markers were sought.

MATERIALS AND METHODS

Study Population

Twenty-nine stable patients receiving PD for at least 6 months and under follow-up in our PD unit were enrolled in our study together with 13 healthy volunteers so as to compare levels of immune cells in the circulation. Exclusion criteria included a history of malignancy, autoimmune disease, current treatment with immunosuppressive medications and chronic infections. Additionally, patients with a recent (<3 months) infection or major adverse cardiovascular event were excluded from the study (**Figure 1**). The comorbidities of all the patients including presence of diabetes mellitus (DM), CAD, peripheral artery disease (PAD) and HF were recorded by evaluation of their medical records. All patients provided signed informed consent. The study protocol was approved by the Ethical Committee of our hospital (5/26-3-2020) and has been registered on ClinicalTrials.gov (NCT04286477).

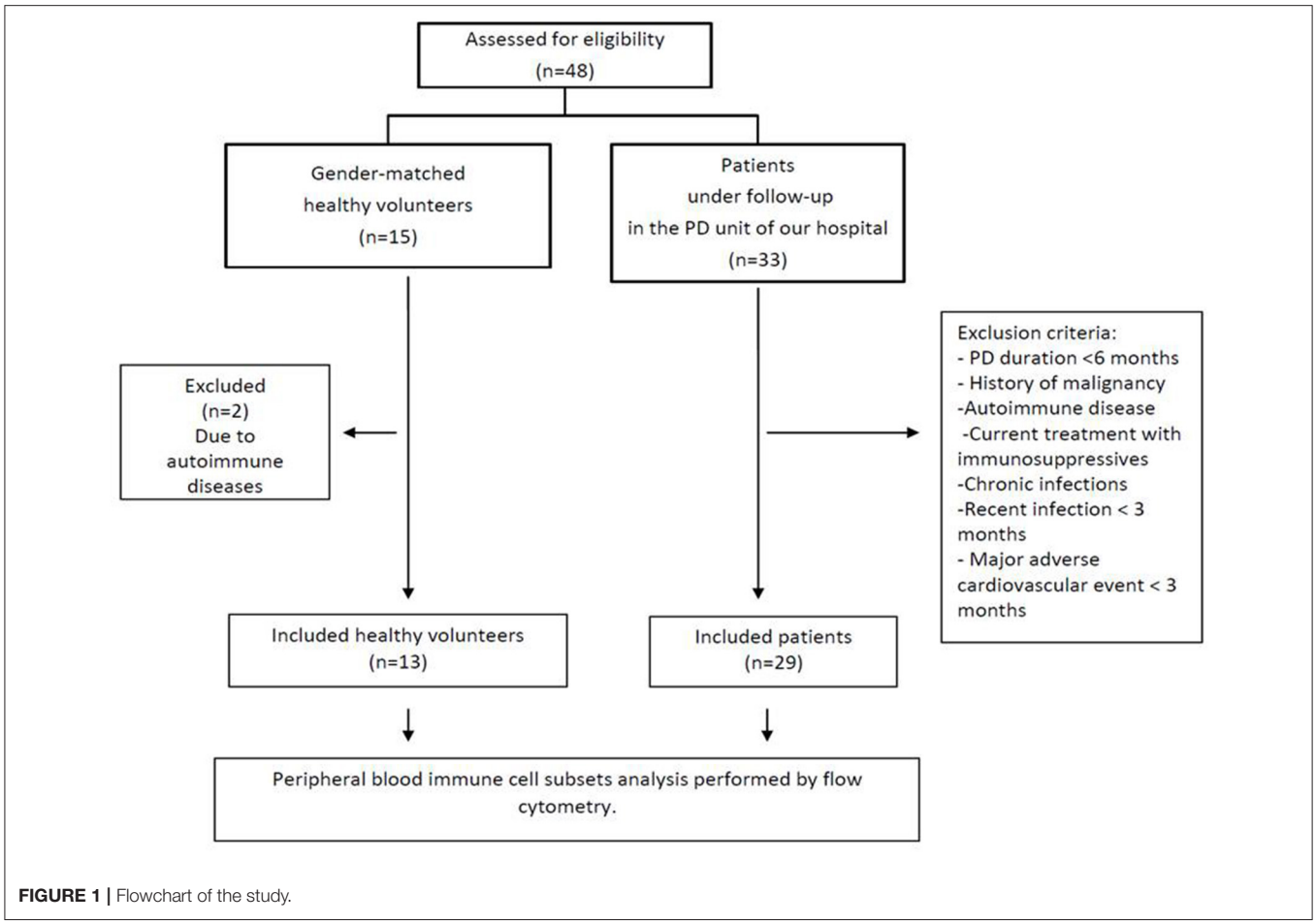


FIGURE 1 | Flowchart of the study.

Laboratory Methods

The peripheral blood immune cell subsets analysis was performed by flow cytometry (FC) in a whole-blood assay using 100 μl of whole blood, within 8 h from blood sample withdrawal. Ethylenediaminetetraacetic acid (EDTA) blood-collecting tubes were used for the collection of 2 ml of whole-blood samples from patients. The following monoclonal antibodies were used for analysis: CD45(BD), CD14(BD), CD16(BD), CD4(BD), CD8(BD), CD56(BD), CD3(BD), CD19(BD), CD25(BD), and Fox-P3(eBioscience^{TM}). Immune cells subtypes were analyzed using flow cytometry (FACSCalibur) and Cell Quest and FACSDiva software (BD Biosciences). 100 μl of whole-blood was added to flow cytometry tubes and incubated with respective antibodies according to manufacturer's instructions. 500 μl of Versalyse (Beckman Coulter) was added and incubated for 10 min at room temperature (18–25°C) protected from light, to lyse red blood cells. Samples were processed immediately for flow cytometry analysis. The data were analyzed using the CellQuest V3.1 software (Becton Dickinson). Accordingly, CD14++CD16-, CD14++CD16+, and CD16+ percentage and absolute number of cells out of the total monocytes were measured. Additionally, NK cells (CD3+CD16+56+), CD3-CD19+ B lymphocytes, CD3+ CD4+ T cells, CD3+CD8+ T cells, and Tregs (CD4+CD25+ FoxP3+) absolute values and percentage out of the total lymphocytes were measured (**Figure 2**). Blood was drawn from all subjects under standardized conditions samples were analyzed using standard techniques. Complete blood counts with differential counts of the white blood cell and conventional inflammatory markers including C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and fibrinogen were measured. Furthermore, serum levels of total protein, albumin, total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, calcium, phosphorus, intact parathyroid hormone (iPTH), 25(OH)-vitamin D and ferritin were also determined. High sensitivity troponin I (hsTnI) was measured as a subclinical index of myocardial damage.

All patients underwent bioimpedance analysis of body composition and fluid status using Fresenius Body Composition Monitor (BCM) for determination of overhydration (OH), extracellular water (ECW), intracellular water (ICW), total body water (TBW) content and the OH/ECW index, simultaneously with analysis of immune cell subsets (13). A lung ultrasound examination (Vscan^{TM} GE Healthcare's) was likewise simultaneously performed with estimation of extravascular lung water by counting vertical "comets" or "B-lines and their sum

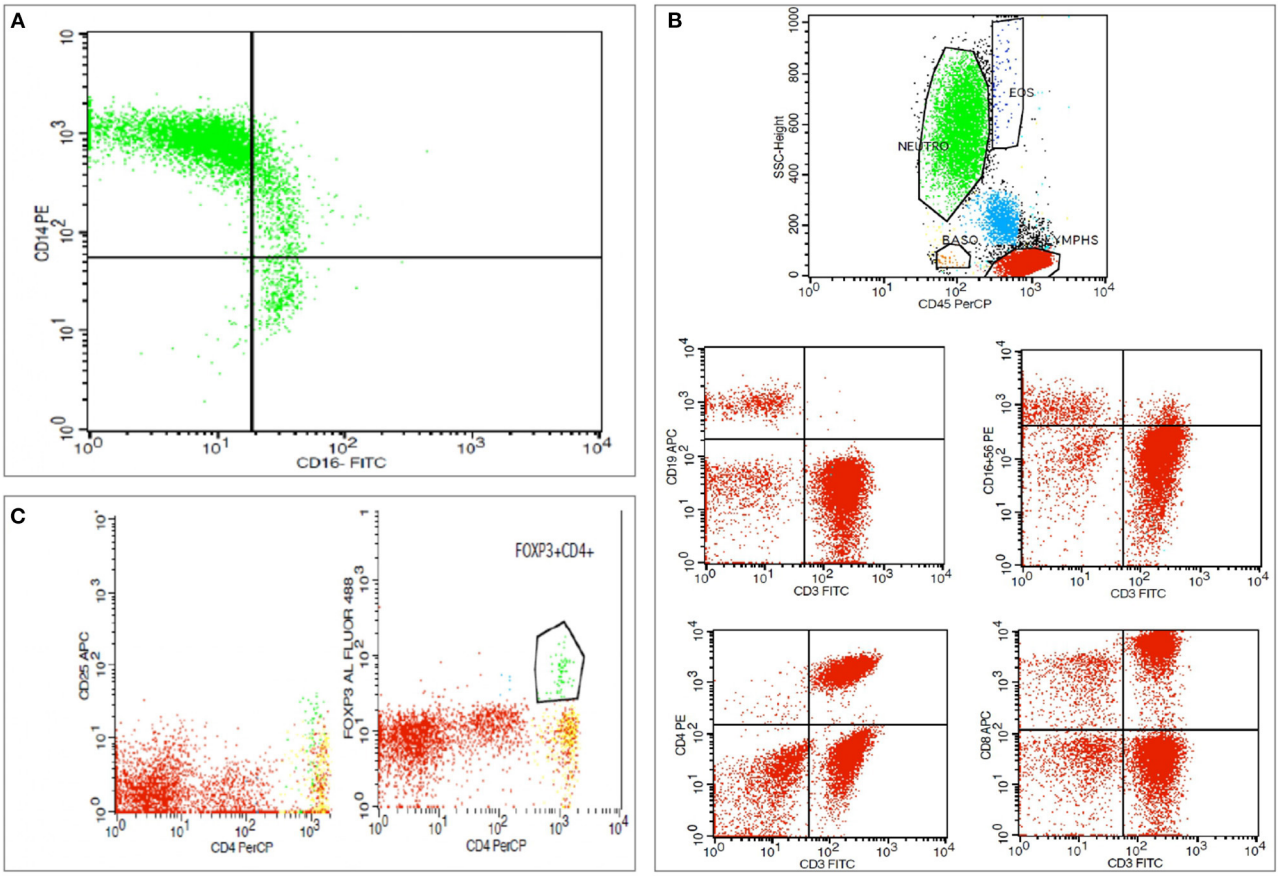


FIGURE 2 | Flow cytometric analysis of a peritoneal dialysis patient. **(A)** Representative dot plots depicting monocyte subsets, differentiated according to their surface expression pattern of CD14 and CD16 in CD14++CD16–, CD14++C16+ and CD14+CD16+ subpopulations. **(B)** Representative dot plots depicting lymphocyte gating with B-lymphocytes, and T lymphocytes, natural killer (NK) cells defined as CD16+CD56+ cells, CD4+ T cells, CD8+ T cells. **(C)** Representative dot plots depicting regulatory T cells (Tregs) defined as CD4+ FoxP3+ $CD25^{high}$ positive cells.

number (14, 15). US-B lines assessment was made in supine position with scanning of the anterior and lateral chest from the second to the fourth intercostal space on the left side and from the second to the fifth intercostal space on the right side, at the parasternal to midaxillary lines as already defined by previous studies (14).

In addition, echocardiographic data from ultrasounds performed within 1 month from immune cell subset analysis were recorded, including left ventricular mass (LVM) and left ventricular mass index (LVMI), left ventricular ejection fraction (EF) and E/E' ratio. Residual renal function (eGFR) defined as the urinary clearance of urea in ml/min and PD adequacy expressed as weekly renal plus peritoneal KT/V of urea, peritoneal transport characteristics as determined by performance of modified PET (16), as well as the normalized protein catabolic rate (nPCR) were recorded.

Statistical Analysis

Descriptive statistics are reported as means ± standard deviations in normally distributed continuous variables, medians and interquantile range in skewed continuous variables and percentages in dichotomous variables. Normal distribution of all continuous variables was tested with the parametric Shapiro-Wilk normality test. Box cox transformation was applied to transform skewed variables (inverse, or log-transformation). In cases normality was not achieved by any transformation variable was transformed to dichotomous using median as cut-off. Differences between cases and controls were assessed by independent samples *t*-test or non-parametric Mann Whitney test, in normally and skewed continuous variables, respectively. Differences between categorical variables were estimated using 2x2 tables and applying chi-square or fisher's exact test, when applicable.

Univariate analysis was performed for any variable of interest. Any variable having a significant univariate test at a significance level of 0.1 was selected as a candidate for the multivariate analysis in order to identify independent predictors of the dependent variable. In the iterative process of variable selection in multivariate analysis, covariates were removed from the model if they were non-significant or not confounders. Linear regression or logistic regression

TABLE 1 | Laboratory, lung ultrasound, bioimpedance and echocardiographic data of the PD patients.

Hemoglobin (g/dl)	10.9 (10.2, 11.8)
Cholesterol (mg/dl)	176.4 ± 40.5
TRG (mg/dl)	167 (110, 254)
HDL (mg/dl)	40.5 (34.0, 47.5)
LDL (mg/dl)	94.1 ± 33.2
Albumin(g/dl)	3.5 ± 0.4
Phosphate (mg/dl)	4.3 ± 0.8
iPTH (pg/ml)	235 (127, 306)
vitD (ng/ml)	8.7 ± 2.8
CRP (mg/L)	3 (2, 5)
Fibrinogen (mg/dl)	517 (453, 559)
hsTroponin (ng/ml)	12.6 (6.5, 20.0)
Ferritin(ng/ml)	215 (95, 476)
D/P creatinine	0.73 ± 0.1
KT/V	2.0 ± 0.4
RRF(ml/min)	4.5 (2.3, 6.3)
nPCR (g/kg/day)	0.86 ± 0.16
ECW (L)	15.9 ± 3.3
TBW (L)	33.4 ± 6.6
OH/ECW	0.12 (−0.25, 0.95)
Lung comets ≥ 2	11 (38%)
LVM (g)	218.2 ± 97.8
LVMI (g/m2)	130.5 ± 41.3
E/E'	10.7 ± 4.3
EF %	60 (47.5, 65.0)

Values are expressed in mean (±SD) or median(IQR 25–75th percentiles). D/P creatinine, dialysate to plasma ratio of creatinine; EF, ejection fraction; ECW, extracellular water; iPTH, intact parathormone; LVM, left ventricular mass; LVMI, left ventricular mass index; nPCR, normalized protein catabolic rate; OH, overhydration; RRF, residual renal function; TBW, total body water.

analysis was used when applicable. Analysis was performed by STATA package, version 14.2 (StataCorp, College Station, TX).

RESULTS

The main laboratory, echocardiographic and bioimpedance analysis data of the 29 PD patients enrolled are presented in **Table 1**. The mean age of the study cohort was 64 years ± 14.3 and 58.6% were males. The median dialysis vintage was 34.5 months (IQR 3.2–141). Primary renal diseases included diabetic nephropathy (seven patients, 24.14%), IgA nephropathy (five patients, 17.24%), whereas the cause of nephropathy was unknown in 12 patients (41.38%). Ten patients were diabetics, while CAD was present in seven patients (24%), peripheral artery disease (PAD) in seven patients (24%), with 11 (38%) patients overall displaying atherosclerotic cardiovascular disease (ACVD). In addition, five patients had HF (17.2 %) and echocardiographic evidence of left ventricular hypertrophy (LVH) was present in 20 patients (71.4%).

TABLE 2 | Immune cell subpopulations in the control and PD group.

	Normal controls	PD patients	*P*-value
WBC	7368.4 ± 1584.4	7451.7 ± 2880.1	0.92
Monocytes	6.4 ± 1.07	6.29 ± 2.02	0.86
CD14++CD16-	88.8 (85.7, 92.5)	87.9 (79.2, 0 90)	0.32
CD14+CD16++	4.11 (2.08, 4.88)	4 (2.5, 6.22)	0.87
CD14++CD16+	4.75 ± 2.75	9.28 ± 6.36	0.002
Lymphocytes (%)	31.3 ± 5.54	22.3 ± 6.28	<0.001
T-lymphocytes (%)	76.09 ± 7.11	76.8 ± 10.1	0.82
B-lymphocytes (%)	9.72 ± 3.63	6.39 ± 3.75	0.01
NK cells (%)	13.2 (8.62, 18.1)	15.4 (9.67, 20.5)	0.54
Tregs (%)	1.85 (1.48, 2.45)	1.79 (1.34, 2.62)	0.71
CD4+ T cells (%)	46.8 ± 7.65	50.1 ± 12.5	0.39
CD8+ T cells (%)	28.9 ± 9.24	25.6 ± 11.2	0.35
CD4CD8 ratio	1.74 (1.19, 2.24)	1.91 (1.40, 3.18)	0.36

Values are expressed in mean (±SD) or median (IQR 25–75th percentiles). CD, cluster of differentiation; NK, natural killer; Tregs, T regulatory cells.

Distribution of the Immune Cell Subpopulations in the PD and Control Groups

Overall, PD patients had 527 ± 199 monocytes and 1731 ± 489 lymphocytes while mean percentage of CD14++CD16+ monocytes was 9.3 ±6.36% (normal range 2–8%), NK cells 16.6 ± 10.3% (normal range 5–15%) and Tregs 2.1 ± 1.76% (normal range 1–3%). **Table 2** depicts the measurements results of the immune cell subpopulations in our cohort and the control group.

Following comparison of the immune cell subpopulations of the PD patients with the control group, we found that PD patients had lower overall total lymphocytes and B-lymphocytes as well as higher CD14++CD16+ monocytes numbers (**Figure 3**).

Correlations of Immune Cell Subpopulations With Clinical Characteristics, Peritoneal Transport Status and Inflammatory Markers of PD Patients

We sought to determine potential correlations between immune cells subpopulations and other clinical characteristics and laboratory parameters in PD patients. Accordingly, patients with higher NK cell levels (>15.4%, $n = 15$) were more likely to be rapid transporters in the modified PET test (D/P creatinine 0.76 ± 0.1 vs. 0.69 ± 0.08, $p = 0.04$). Additionally, patients with higher NK cell levels (>15.4%) had higher cholesterol levels (191.2 ± 47.1mg/dl vs. 160.64 ± 25.1mg/dl, $p = 0.03$) as well as higher CRP levels [2.5 (2, 5) mg/L vs. 5 (3, 9) mg/L, $p = 0.06$]. However, in multiple logistic regression analysis, only the D/P creatinine ratio (odds ratio 7.5; 95% confidence interval, 1.13–50.01; $p = 0.036$) and the total cholesterol levels (odds ratio 1.09; 95% confidence interval (CI), 1.01–1.18; $p = 0.027$) remained significant independent predictors of NK levels.

Regarding Tregs, significant correlations were found between Tregs levels with age and the nPCR, with patients displaying

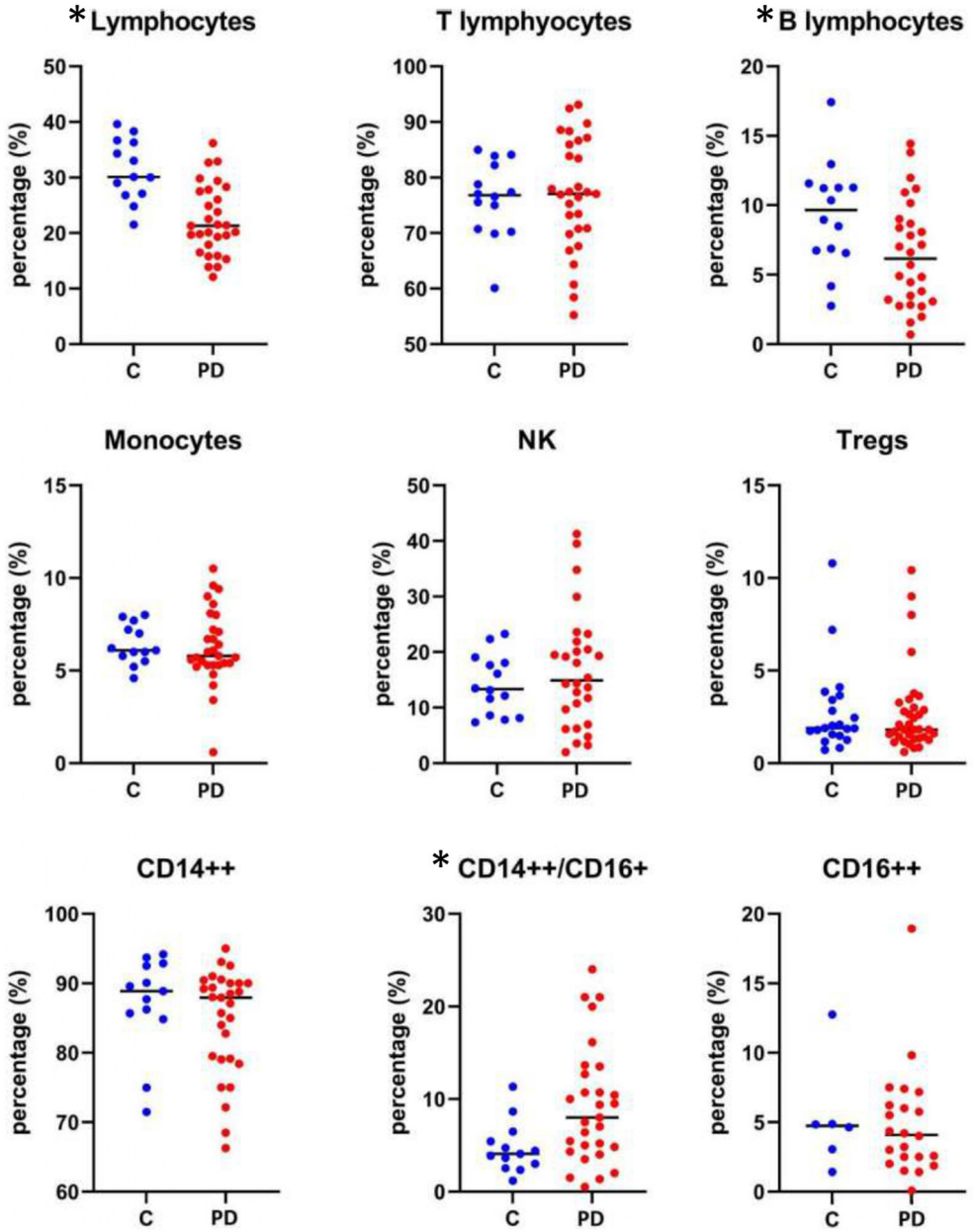


FIGURE 3 | Immune cells subpopulations counts in control and PD patients. *$p < 0.05$, Values are expressed as mean or medians.

higher percentage of Tregs (>1.79%) being older (70.8 ± 10.7 years vs. 57.7 ± 14.7years, $p = 0.011$) and having a higher nPCR (0.83 ± 0.14 vs. 0.91 ± 0.17, $p = 0.09$).

Correlations of Immune Cell Subpopulations in PD Patients With Indices of Overhydration and Phenotypes of CVD

With regard to monocytes subtypes, an inverse correlation was detected between CD14++CD16+ % levels and the presence of ACVD (β-coefficient = −5.57, $p = 0.019$). Patients with higher NK cell levels had a higher prevalence of CAD (40 vs. 28.6%, $p = 0.039$) as well as higher E/E'ratios in cardiac ultrasound (12.57 ± 4.34 vs. 8.78 ± 3.53, $p = 0.02$). Patients with higher percentage of Tregs (>1.79%) were more likely to manifest LVH (92.8 vs. 50%, $p = 0.012$), a correlation however which was not maintained following multiple regression analysis.

Regarding phenotypes of CVD, patients with prevalent CAD in comparison to patients without known CAD were diabetics (71.4 vs. 22.7%, $p = 0.018$), had higher CRP, fibrinogen and albumin levels [9 mg/L (5,38), 576 mg/dl (544,737), 3.5 g/dl (3.4,3.7) vs. 3 mg/L (2,4), $p = 0.0004$, 486.5 mg/dl (444,530), p

= 0.004, 3.1 g/dl (3,3.4), $p = 0.005$], respectively and had a higher E/E' ratio in heart ultrasound (14.08 ± 5.42 vs. 9.65 ± 3.49, $p = 0.018$). Additionally, PD patients with prevalent CAD had NK cells levels elevated above median values (85.7% vs. 40.9%, $p = 0.04$) as well as a lower percentage of B cells (3.85 ± 2.46 vs. 7.2 ± 3.77%, $p = 0.03$). In multiple logistic regression analysis, the percentage of NK levels and of B cells remained an independent significant predictor of the presence of CAD. Thus, patients with increased NK cell levels (>15.4%) had 3.8 times higher risk of CAD comparing with patients with lower NK cell levels (95% CI, 1.86–77.87; $p = 0.034$). On the other hand, the percentage of B cells was inversely associated with the presence of CAD (increase of B-lymphocyte by 1% was independently associated with 30% less risk for presence of CAD (95% CI, −0.71–0.01; $p = 0.05$).

Peritoneal dialysis patients with ACVD as compared to patients without ACVD ($n = 18$) were older (71.2 ± 8.81 years vs. 59.6 ± 15.5, $p = 0.03$) and mainly diabetics (63.6 vs. 16.6%, $p = 0.01$), had lower serum albumin and LDL levels (3.3 ± 2.78 g/dl, 78.8 ± 29.2 mg/dl vs. 3.61 ± 0.37g/dl, $p = 0.02$, 103.7 ± 32.7 md/dl $p = 0.06$, respectively), higher CRP levels [6 mg/L (3,9) vs. 3mg/L (2,4), $p = 0.01$] and displayed higher E/E' ratio in cardiac ultrasound (13.5 ± 5.21 vs. 9.16 ± 2.94, $p = 0.01$). The percentages of CD14++CD16- monocytes and Tregs were significantly higher in patients with ACVD [88.4 ± 8.67 vs. 82.2 ± 8.44, $p = 0.02$ and 1.82 (1.71, 3.45) vs. 1.49 (1.26, 2.08), $p = 0.01$, respectively] while the percentage of CD14++CD16+ monocytes was lower in this patient group [5.2 (2.0, 7.5) vs. 7.49 (4.84, 10.15), $p = 0.017$]. In multiple logistic regression analysis, apart from presence of DM as well as serum albumin and CRP values, the percentages of the CD14++CD16+ monocytes and the Tregs were significantly associated with the presence of ACVD; increase of CD14++CD16+ up to 1% was associated with 31% less risk for ACVD (OR 0.69; 95% CI, 0.48–0.98; $p = 0.041$) and increase of Tregs up to 1% was associated with 20 times higher risk for ACVD (OR 20.5; 95% CI, 1.5–274.7; $p = 0.022$).

Additionally, we examined if patients with evidence of overhydration (defined by the presence of lung comets in lung ultrasound) had different characteristics and expression of immune cell subpopulations as compared with euvolemic patients. Eleven overhydrated patients as defined by presence of ≥2 lung comets showed evidence of overhydration in bioimpedance measurements [ECV/TBW 0.51 (0.49, 0.53) vs. 0.47 (0.43, 0.50), $p = 0.03$)] as well. Patients with clinical evidence of overhydration as compared to euvolaemic patients, had higher CRP, fibrinogen and hsTnI levels [6 mg/L (5,11), 544 mg/dl (511, 721), 17.4 ng/ml (13.1, 42.7) vs. 3 mg/L (2, 4), $p = 0.004$, 486 mg/dl (422, 549), $p = 0.05$, 10.35 ng/ml (4.7, 13.8), $p = 0.03$, respectively] as well as higher E/E'ratios [13 (11.8, 15) vs. 8.4 (7, 11), $p = 0.02$]. Patients with lower CD14++CD16+ % levels had higher OH/ECV values in bioimpedance analysis (β-coefficient = −0.037, $p = 0.042$). In addition, overhydrated patients had lower percentages of lymphocytes (18.3 ± 4.29% vs. 24.7 ± 6.18%, $p = 0.006$) and higher percentages of NK cells [20.5% (14.3, 23.6) vs. 13.21% (6.23, 19.2), $p = 0.04$)]. In multiple logistic regression analysis the CRP [for every increase of 1 mg/dL, there was 1.43 times higher risk for presence of lung comets (OR 1.43; 95% CI, 1.00–2.05; $p = 0.04$)] and the percentage of lymphocytes [a decrease of 1% is associated with 21% less risk for lung comets (OR 0.79; 95% CI, 0.63–0.99; $p = 0.04$)] were independently associated with the presence of lung comets.

DISCUSSION

There are scarce data in the literature regarding the expression of specific immune cell subtypes, including the CD14++CD16+ proinflammatory monocyte subpopulation, NK cells and Tregs in patients undergoing PD. In addition the potential associations of immune cells with the indices of dialysis adequacy and overhydration as well as the phenotypes of prevalent cardiovascular disease have not been studied in this patient population until now.

The results of our study showed that patients undergoing PD display elevated levels of the pro-inflammatory CD14++CD16+ monocyte subset as compared to normal individuals, indicating the persistence of the inflammatory milieu in this population. Our findings confirm results from previous studies showing that both hemodialysis and PD patients have increased counts of $CD14^{++}CD16^{+}$ monocytes compared to individuals without CKD (17). On the other hand, we found an inverse correlation of CD14++CD16+ levels with presence of ACVD, although longitudinal epidemiological studies have confirmed at large a direct relationship between increased CD14++CD16+ monocytes and occurrence of adverse cardiovascular outcomes in patients with CKD and dialysis patients (18, 19). However, it should be noted that the number of peritoneal dialysis patients evaluated by these studies was very small, with only one study including <20 peritoneal dialysis patients (17). Our finding support a suggested J-shaped relationship that might exist between CD16+ monocyte subsets and adverse outcomes in patients receiving hemodialysis, such that both high and low CD16+ counts confer an increased risk of all-cause and cardiovascular mortality (20). Moreover, PD patients with ACVD were found to have higher levels of classical CD14++CD16- monocytes. It should be noted that available data in the literature remain controversial with regard to the specific status and role of the classical monocytes in patients with ACVD with or without CKD (20–23).

The multifaceted nature of NK cells and their role in the propagation vs. modulation of inflammation remains a subject of dispute. In addition, it should be noted that inflammation itself has been associated both with induction of NK cell apoptosis and augmented proliferation in the setting of cytokine stimulation. Increased NK cell levels in the circulation have been associated with disease activity or adverse prognosis in several disease models of inflammation, such as sepsis and autoimmune disease (24–27). Accordingly, both in experimental sepsis models and in clinical studies of patients with sepsis and septic shock, NK cells in the circulation increased in numbers and displayed an activated phenotype whereas their counts showed a direct association with mortality (24–26).

Furthermore, faster peritoneal transport status in PD patients has been associated among others with intraperitoneal and

systemic inflammation. We have found a direct correlation between increased NK cell counts and fast peritoneal transport status in our cohort (28). Moreover, available data suggest that fluid overload is significantly and reciprocally associated with systemic microinflammation and it is more frequent in fast trasporters (29). Our study results indicate that increased NK cells were linked to fluid overload in PD patients, determined either as overhydration in lung ultrasound, BCM measurements or as an increased E/E' in heart ultrasound.

Although NK cells are suspected to play a direct role in atherogenesis considering their abundance in the necrotic cores of atherosclerotic plaques, it remains controversial whether they are harmful or protective toward the vascular tissues (30). Experimental models have shown that depletion of functional NK cells decreases the atherosclerosis burden in atherosclerosis-susceptible LDL receptor null mice (31). On the other hand, a recent study in mice, lacking or having hyper-responsive NK cells, showed that the atherosclerotic burden in the aortic sinus and in the descending aorta did not change, thus suggesting that these cells have no effect on the pathogenesis of atherosclerosis (32). In our study, increased NK cell levels in PD patients were associated with increased risk for prevalent CAD. Although some clinical studies have found reduced NK cell counts and cytotoxic activity in patients with prevalent CAD, others have shown not only increased levels of total circulating NK cells in atherosclerotic patients but a direct relationship between NK cell counts and complications in these patients as well (6, 33–35).

Finally, we did not detect any significant differences in NK cell counts between PD patients and healthy subjects. Previous studies have yielded controversial results regarding NK cell counts in patients undergoing hemodialysis or peritoneal dialysis (36–38). However, it has been suggested that lower NK cell counts directly correlate with the glomerular filtration rate (GFR) in hemodialysis patients, thus allowing us to speculate that the preservation of residual renal function as occurs in PD, might have affected our results (36).

Our results confirm results of earlier studies regarding total lymphocytes and B-lymphocyte depletion due to an increased apoptosis in patients with ESKD undergoing dialysis (39, 40). In addition, we found an inverse association of the total lymphocytes count and percentage of B cells with overhydration and the presence of CAD respectively in PD patients. Reduced total lymphocyte count is an established independent predictor of mortality in heart failure patients whereas with regard to atherosclerosis, the mode that B lymphocytes affect the atherosclerotic lesions currently remains a subject of ongoing investigation (41, 42). Likewise, $CD19^{+}$ B-cell lymphopenia has been suggested as an independent predictor of all-cause and CV mortality in hemodialysis patients (40).

With regard to Tregs, the influence of dialysis on their counts and function remains to be further clarified (43–45). Thus, a recent meta-analysis showed that ESKD patients not undergoing dialysis displayed a lower percentage of Tregs on CD4+ T-cells compared to healthy individuals, but on the other hand no significant difference was observed with respect to Tregs percentage between hemodialysis patients and healthy individuals (44). We found no significant differences between the percentage of Tregs on total lymphocytes and normal controls. A great deal of experimental and clinical evidence indicates a beneficial cardioprotective role of Tregs, associating their reduced numbers and impaired function with various models of cardiovascular diseases, including atherosclerosis, hypertension and heart failure (46). On the other hand, we found that patients with increased percentage of Tregs were more likely to be older and have LVH or ACVD. Whether this finding should be ascribed to a compensatory mechanism or specific immunologic properties of the Tregs themselves remains to be elucidated by future studies. Similarly, a study investigating whether the levels of circulating Treg cells relate to the degree of atherosclerosis showed an increase in Tregs only in patients with acute coronary syndromes, whereas patients with stable angina Tregs we not altered compared to healthy control subjects (47). In addition, no difference in regulatory T cells was observed between type 2 diabetes mellitus patients with cardiovascular disease as compared to those without (48).

To our knowledge, this is the first study to evaluate the association of the profiles of immune cells subpopulations with peritoneal transport characteristics, indices of overhydration and phenotypes of cardiovascular disease in a cohort of long-term PD patients. Yet, there are limitations to our study, including a relatively small sample size as well as its observational and cross-sectional nature. Moreover, a relatively small number of the patients included had prevalent CAD or ACVD and overt overhydration. Finally, only the phenotypes of immune cell subpopulations were studied but not their function or association with other immune markers, which is the aim of another study that our group is currently conducting.

The state of pro-inflammation and immune deregulation appear to persist after initiating PD. Future research is required to evaluate the role of immune cells subsets as potential tools to identify patients who are at the highest risk for complications and to guide interventions that may improve clinical outcomes.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University Hospital of Ioannina Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All the authors have substantially contributed to the conception, design, and research conduct as well as to the writing of the manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Tripepi G, Mallamaci F, Zoccali C. Inflammation markers, adhesion molecules, and all-cause and cardiovascular mortality in patients with ESRD: searching for the best risk marker by multivariate modeling. *J Am Soc Nephrol.* (2005) 16:S83–8. doi: 10.1681/ASN.2004110972
2. Dounousi E, Duni A, Naka KK, Vartholomatos G, Zoccali C. The innate immune system and cardiovascular disease in ESKD: monocytes and natural killer cells. *Curr Vasc Pharmacol.* (2021) 19:63–76. doi: 10.2174/1570161118666200628024027
3. Stansfield BK, Ingram DA. Clinical significance of monocyte heterogeneity. *Clin Transl Med.* (2015) 4:5. doi: 10.1186/s40169-014-0040-3
4. Rogacev KS, Cremers B, Zawada AM, Seiler S, Binder N, Ege P, et al. CD14++CD16+ monocytes independently predict cardiovascular events: a cohort study of 951 patients referred for elective coronary angiography. *J Am Coll Cardiol.* (2012) 60:1512–20. doi: 10.1016/j.jacc.2012.07.019
5. Michel T, Poli A, Cuapio A, Briquemont B, Iserentant G, Ollert M, et al. Human CD56bright NK cells: an update. *J Immunol.* (2016) 196:2923–31. doi: 10.4049/jimmunol.1502570
6. Bacteman K, Ernerudh J, Jonasson L. Natural killer (NK) cell deficit in coronary artery disease: no aberrations in phenotype but sustained reduction of NK cells is associated with low-grade inflammation. *Clin Exp Immunol.* (2014) 175:104–12. doi: 10.1111/cei.12210
7. Zhuang R, Feinberg MW. Regulatory T cells in ischemic cardiovascular injury and repair. *J Mol Cell Cardiol.* (2020) 147:1–11. doi: 10.1016/j.yjmcc.2020.08.004
8. Cobo G, Lindholm B, Stenvinkel P. Chronic inflammation in end stage renal disease and dialysis. *Nephrol Dial Transplant.* (2018) 33(suppl_3):iii35–40. doi: 10.1093/ndt/gfy175
9. Li PK, Ng JK, Mcintyre CW. Inflammation and peritoneal dialysis. *Semin Nephrol.* (2017) 37:54–65. doi: 10.1016/j.semnephrol.2016.10.007
10. Liu SH, Li YJ, Wu HH, Lee CC, Lin CY, Weng CH, et al. High-sensitivity C-reactive protein predicts mortality and technique failure in peritoneal dialysis patients. *PLoS ONE.* (2014) 9:e93063. doi: 10.1371/journal.pone.0093063
11. Cho Y, Johnson DW, Vesey DA, Hawley CM, Pascoe EM, Clarke M, et al. Baseline serum interleukin-6 predicts cardio- vascular events in incident peritoneal dialysis patients. *Perit Dial Int.* (2015) 35:35–42. doi: 10.3747/pdi.2013.00272
12. Aufricht C, Beelen R, Eberl M, Fischbach M, Fraser D, Jörres A, et al. Biomarker research to improve clinical outcomes of peritoneal dialysis: consensus of the European training and research in peritoneal dialysis (EuTRiPD) network. *Kidney Int.* (2017) 92:824–35. doi: 10.1016/j.kint.2017.02.037
13. Alexandrou ME, Balafa O, Sarafidis P. Assessment of hydration status in peritoneal dialysis patients: validity, prognostic value, strengths, and limitations of available techniques. *Am J Nephrol.* (2020) 51:589–612. doi: 10.1159/000509115
14. Mallamaci F, Benedetto FA, Tripepi R, Rastelli S, Castellino P, Tripepi G, et al. Detection of pulmonary congestion by chest ultrasound in dialysis patients. *JACC Cardiovasc Imaging.* (2010) 3:586–94. doi: 10.1016/j.jcmg.2010.02.005
15. Panuccio V, Enia G, Tripepi R, Torino C, Garozzo M, Battaglia GG, et al. Chest ultrasound and hidden lung congestion in peritoneal dialysis patients. *Nephrol Dial Transplant.* (2012) 27:3601–5. doi: 10.1093/ndt/gfs116
16. Smit W, van Dijk P, Langedijk MJ, Schouten N, van den Berg N, Struijk DG, et al. Peritoneal function and assessment of reference values using a 3.86% glucose solution. *Perit Dial Int.* (2003) 23:440–9. doi: 10.1177/089686080302300507
17. Heine GH, Ulrich C, Seibert E, Seiler S, Marell J, Reichart B, et al. CD14(++)CD16+ monocytes but not total monocyte numbers predict cardiovascular events in dialysis patients. *Kidney Int.* (2008) 73:622–9. doi: 10.1038/sj.ki.5002744
18. Rogacev KS, Seiler S, Zawada AM, Reichart B, Herath E, Roth D, et al. CD14++CD16+ monocytes and cardiovascular outcome in patients with chronic kidney disease. *Eur Heart J.* (2011) 32:84–92. doi: 10.1093/eurheartj/ehq371
19. Heine GH, Ortiz A, Massy ZA, Lindholm B, Wiecek A, Martínez-Castelao A, et al. Monocyte subpopulations and cardiovascular risk in chronic kidney disease. *Nat Rev Nephrol.* (2012) 8:362–9. doi: 10.1038/nrneph.2012.41
20. Jeng Y, Lim PS, Wu MY, Tseng T, Chen CH, Chen HP, et al. Proportions of proinflammatory monocytes are important predictors of mortality risk in hemodialysis patients. *Mediators Inflamm.* (2017) 2017:1070959. doi: 10.1155/2017/1070959
21. Arslan U, Kocaoglu I, Falay MY, Balci M, Duyuler S, Korkmaz A. The association between different monocyte subsets and coronary collateral development. *Coron Artery Dis.* (2012) 23:16–21. doi: 10.1097/MCA.0b013e32834df5b3
22. Tsujioka H, Imanishi T, Ikejima H, Tanimoto T, Kuroi A, Kashiwagi M, et al. Post-reperfusion enhancement of CD14(+)CD16(-) monocytes and microvascular obstruction in ST-segment elevation acute myocardial infarction. *Circ J.* (2010) 74:1175–82. doi: 10.1253/circj.CJ-09-1045
23. Tapp LD, Shantsila E, Wrigley BJ, Pamukcu B, Lip GY. The CD14++CD16+ monocyte subset and monocyte-platelet interactions in patients with ST-elevation myocardial infarction. *J Thromb Haemost.* (2012) 10:1231–41. doi: 10.1111/j.1538-7836.2011.04603.x
24. Guo Y, Patil NK, Luan L, Bohannon JK, Sherwood ER. The biology of natural killer cells during sepsis. *Immunology.* (2018) 153:190–202. doi: 10.1111/imm.12854
25. Andaluz-Ojeda D, Iglesias V, Bobillo F, Almansa R, Rico L, Gandía F, et al. Early natural killer cell counts in blood predict mortality in severe sepsis. *Crit Care.* (2011) 15:R243. doi: 10.1186/cc10501
26. Andaluz-Ojeda D, Iglesias V, Bobillo F, Nocito M, Loma AM, Nieto C, et al. Early levels in blood of immunoglobulin M and natural killer cells predict outcome in nonseptic critically ill patients. *J Crit Care.* (2013) 28:1110.e7–10. doi: 10.1016/j.jcrc.2013.06.007
27. Liu M, Liang S, Zhang C. NK cells in autoimmune diseases: protective or pathogenic? *Front Immunol.* (2021) 12:624687. doi: 10.3389/fimmu.2021.624687
28. Davies SJ. Peritoneal solute transport and inflammation. *Am J Kidney Dis.* (2014) 64:978–86. doi: 10.1053/j.ajkd.2014.06.030
29. Hung SC, Kuo KL, Peng CH, Wu CH, Lien YC, Wang YC, et al. Volume overload correlates with cardiovascular risk factors in patients with chronic kidney disease. *Kidney Int.* (2014) 85:703–9. doi: 10.1038/ki.2013.336
30. Selathurai A, Deswaerte V, Kanellakis P, Tipping P, Toh B, Bobik A, et al. Natural killer (NK) cells augment atherosclerosis by cytotoxic-dependent mechanisms. *Cardiovasc Res.* (2014) 102:128–37. doi: 10.1093/cvr/cvu016
31. Whitman SC, Rateri DL, Szilvassy SJ, Yokoyama W, Daugherty A. Depletion of natural killer cell function decreases atherosclerosis in low-density lipoprotein receptor null mice. *Arterioscler Thromb Vasc Biol.* (2004) 24:1049–54. doi: 10.1161/01.ATV.0000124923.95545.2c
32. Nour-Eldine W, Joffre J, Zibara K, Esposito B, Giraud A, Zeboudj L, et al. Genetic depletion or hyperresponsiveness of natural killer cells do not affect atherosclerosis development. *Circ Res.* (2018) 122:47–57. doi: 10.1161/CIRCRESAHA.117.311743
33. Jonasson L, Backteman K, Ernerudh J. Loss of natural killer cell activity in patients with coronary artery disease. *Atherosclerosis.* (2005) 183:316–21. doi: 10.1016/j.atherosclerosis.2005.03.011
34. Bruunsgaard H, Pedersen AN, Schroll M, Skinhoj P, Pedersen BK. Decreased natural killer cell activity is associated with atherosclerosis in elderly humans. *Exp Gerontol.* (2001) 37:127–36. doi: 10.1016/S0531-5565(01)00162-0
35. Kotfis K, Biernawska J, Zegan-Baranska M, Zukowski M. Peripheral blood lymphocyte subsets ($CD4^+$, $CD8^+$ T Cells, NK Cells) in patients with cardiovascular and neurological complications after carotid endarterectomy. *Int J Mol Sci.* (2015) 16:10077–94. doi: 10.3390/ijms160510077
36. Vacher-Coponat H, Brunet C, Lyonnet L, Bonnet E, Loundou A, Sampol J, et al. Natural killer cell alterations correlate with loss of renal function and dialysis duration in uraemic patients. *Nephrol Dial Transplant.* (2008) 23:406–14. doi: 10.1093/ndt/gfm596
37. Leander M, Grzegorzewska AE. Natural killer cells in continuous ambulatory peritoneal dialysis patients. *Nephrol Dial Transplant.* (2009) 24:1696–7. doi: 10.1093/ndt/gfp097
38. Griveas I, Visvardis G, Papadopoulou D, Mitsopoulos E, Kyriklidou P, Manou E, et al. Cellular immunity and levels of parathyroid hormone

in uremic patients receiving hemodialysis. *Ren Fail.* (2005) 27:275–8. doi: 10.1081/JDI-200056616
39. Pahl MV, Gollapudi S, Sepassi L, Gollapudi P, Elahimehr R, Vaziri ND. Effect of end-stage renal disease on B-lymphocyte subpopulations, IL-7, BAFF and BAFF receptor expression. *Nephrol Dial Transplant.* (2010) 25:205–12. doi: 10.1093/ndt/gfp397
40. Molina M, Allende LM, Ramos LE, Eduardo Gutiérrez E, Pleguezuelo DE, Hernández ER, et al. CD19 $^{+}$ B-Cells, a New Biomarker of Mortality in Hemodialysis Patients. *Front Immunol.* (2018) 9:1221. doi: 10.3389/fimmu.2018.01221
41. Vaduganathan M, Ambrosy AP, Greene SJ, Mentz RJ, Subacius HP, Maggioni AP, et al. Predictive value of low relative lymphocyte count in patients hospitalized for heart failure with reduced ejection fraction: insights from the EVEREST trial. *Circ Heart Fail.* (2012) 5:750–8. doi: 10.1161/CIRCHEARTFAILURE.112.970525
42. Sage AP, Tsiantoulas D, Binder CJ, Mallat Z. The role of B cells in atherosclerosis. *Nat Rev Cardiol.* (2019) 16:180–96. doi: 10.1038/s41569-018-0106-9
43. Afzali B, Edozie FC, Fazekasova H, Scottà C, Mitchell PJ, Canavan JB, et al. Comparison of regulatory T cells in hemodialysis patients and healthy controls: implications for cell therapy in transplantation. *Clin J Am Soc Nephrol.* (2013) 8:1396–405. doi: 10.2215/CJN.12931212
44. Caprara C, Kinsey GR, Corradi V, Xin W, Ma JZ, Scalzotto E, et al. The influence of hemodialysis on T regulatory cells: a meta-analysis and systematic review. *Blood Purif.* (2016) 42:307–13. doi: 10.1159/000449242
45. Caprara C, Corradi V, Scalzotto E, Frigo AC, Proglio M, Sharma R, et al. Differential effects of peritoneal and hemodialysis on circulating regulatory T cells one month post initiation of renal replacement therapy. *Clin Nephrol.* (2021) 95:37–44. doi: 10.5414/CN110158
46. Meng X, Yang J, Dong M, Zhang K, Tu E, Gao Q, et al. Regulatory T cells in cardiovascular diseases. *Nat Rev Cardiol.* (2016) 13:167–79. doi: 10.1038/nrcardio.2015.169
47. Ammirati E, Cianflone D, Banfi M, Vecchio V, Palini A, De Metrio M, et al. Circulating CD4+CD25hiCD127lo regulatory T-Cell levels do not reflect the extent or severity of carotid and coronary atherosclerosis. *Arterioscler Thromb Vasc Biol.* (2010) 30:1832–41. doi: 10.1161/ATVBAHA.110.206813
48. Rattik S, Engelbertsen E, Wigren M, Ljungcrantz I, Östling G, Persson M, et al. Elevated circulating effector memory T cells but similar levels of regulatory T cells in patients with type 2 diabetes mellitus and cardiovascular disease. *Diab Vasc Dis Res.* (2019) 16:270–80. doi: 10.1177/1479164118817942

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

ORIGINAL RESEARCH
published: 24 December 2021
doi: 10.3389/fmed.2021.796676

Longitudinal Analysis of Antibody Responses to the mRNA BNT162b2 Vaccine in Patients Undergoing Maintenance Hemodialysis: A 6-Month Follow-Up

André Weigert[1,2,3], Marie-Louise Bergman[4], Lígia A. Gonçalves[4], Iolanda Godinho[1,5], Nádia Duarte[4], Rita Abrantes[1,6], Patrícia Borges[4], Ana Brennand[4], Vanessa Malheiro[4], Paula Matoso[4], Onome Akpogheneta[4], Lindsay Kosack[4], Pedro Cruz[1,7], Estela Nogueira[1,5], Magda Pereira[1], Ana Ferreira[1], Marco Marques[8], Telmo Nunes[9], João Faro-Viana[10], Jocelyne Demengeot[4]* and Carlos Penha-Gonçalves[4]*

[1] DaVita Óbidos, Óbidos, Portugal, [2] Serviço de Nefrologia, Centro Hospitalar de Lisboa Ocidental, Hospital Santa Cruz, Carnaxide, Portugal, [3] Faculdade de Medicina, Instituto de Farmacologia e Neurociências, Universidade de Lisboa, Lisboa, Portugal, [4] IGC, Instituto Gulbenkian de Ciência, Oeiras, Portugal, [5] Serviço de Nefrologia e Transplantação Renal, Centro Hospitalar de Lisboa Norte EPE, Lisboa, Portugal, [6] Serviço de Nefrologia, Centro Hospitalar do Médio Tejo, Torres Novas, Portugal, [7] Serviço de Nefrologia, Hospital Das Forças Armadas, Lisboa, Portugal, [8] Affidea Laboratório Lisboa, Lisboa, Portugal, [9] CIISA, Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisboa, Portugal, [10] Serviço de Patologia Clínica, Centro Hospitalar de Lisboa Ocidental EPE, Carnaxide, Portugal

Background: Patients on hemodialysis (HD) are at higher risk for COVID-19, overall are poor responders to vaccines, and were prioritized in the Portuguese vaccination campaign.

Objective: This work aimed at evaluating in HD patients the immunogenicity of BTN162b2 after the two doses induction phase, the persistence of specific antibodies along time, and factors predicting these outcomes.

Methods: We performed a prospective, 6-month long longitudinal cohort analysis of 156 HD patients scheduled to receive BTN162b2. ELISA quantified anti-spike IgG, IgM, and IgA levels in sera were collected every 3 weeks during the induction phase (t0 before vaccine; t1, d21 post first dose; and t2 d21 post second dose), and every 3–4 months during the waning phase (t3, d140, and t4, d180 post first dose). The age-matched control cohort was similarly analyzed from t0 to t2.

Results: Upon exclusion of participants identified as previously exposed to SARS-CoV-2, seroconversion at t1 was lower in patients than controls (29 and 50%, respectively, $p = 0.0014$), while the second vaccine dose served as a boost in both cohorts (91 and 95% positivity, respectively, at t2, $p = 0.2463$). Lower response in patients than controls at t1 was a singularity of the participants ≤70 years ($p = 2.01 \times 10^{-05}$), associated with immunosuppressive therapies ($p = 0.013$), but not with lack of responsiveness to hepatitis B. Anti-spike IgG, IgM, and IgA levels decreased at t3,

OPEN ACCESS

Edited by:
Maria J. Stangou,
Aristotle University of Thessaloniki, Greece

Reviewed by:
Natalia Ortega Herrero,
Instituto Salud Global Barcelona (ISGlobal), Spain
Waleed Mahallawi,
Taibah University, Saudi Arabia
Ana-Luisa Stefanski,
Charité University Medicine Berlin, Germany

***Correspondence:**
André Weigert
andre.weigert@davita.com
Jocelyne Demengeot
jocelyne@igc.gulbenkian.pt

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: 17 October 2021
Accepted: 24 November 2021
Published: 24 December 2021

Citation:
Weigert A, Bergman M-L, Gonçalves LA, Godinho I, Duarte N, Abrantes R, Borges P, Brennand A, Malheiro V, Matoso P, Akpogheneta O, Kosack L, Cruz P, Nogueira E, Pereira M, Ferreira A, Marques M, Nunes T, Faro-Viana J, Demengeot J and Penha-Gonçalves C (2021) Longitudinal Analysis of Antibody Responses to the mRNA BNT162b2 Vaccine in Patients Undergoing Maintenance Hemodialysis: A 6-Month Follow-Up. Front. Med. 8:796676. doi: 10.3389/fmed.2021.796676

with IgG levels further waning at t4 and resulting in >30% seronegativity. Anti-spike IgG levels at t1 and t4 were correlated ($\rho = 0.65$, $p < 2.2 \times 10^{-16}$).

Conclusions: While most HD patients seroconvert upon 2 doses of BNT162b2 vaccination, anti-spike antibodies levels wane over the following 4 months, leading to early seroreversion in a sizeable fraction of the patients. These findings warrant close monitoring of COVID-19 infection in vaccinated HD patients, and advocate for further studies following reinforced vaccination schedules.

Keywords: BNT162b2, chronic hemodialysis, COVID-19, IgG, SARS-CoV-2, vaccine

INTRODUCTION

Patients with chronic kidney disease requiring renal replacement therapy and receiving in-center hemodialysis (HD) treatment are at an increased risk of SARS-Cov-2 infection, and of severe COVID-19 (1). Moreover, HD patients may pose additional stress in the hospital dialysis capacity when admitted, as most receive routine dialysis treatments as outpatients.

End-stage renal disease is simultaneously associated with systemic inflammation (2) and immune deficiency (3). Systemic inflammation contributes to atherosclerosis, cardiovascular disease, cachexia, and anemia, contributing to enhanced susceptibility to severe COVID, whereas immune deficiency leads to impaired response to vaccination and increased incidence and severity of microbial infections. Several studies evidenced abnormal immune response both to viral infection and to vaccination in HD patients (4–6). Blunted antibody responses to influenza (7), pneumococcal (8), and hepatitis B vaccination (9) are indicators of abnormal adaptative immunity in these patients. This lack of response is in part due to uremic toxins that may lead to alterations in B-lymphocyte function, among others (10). Kidney deficiency is associated with vitamin D insufficiency contributing to weakened immunity. Given the impaired antibody response of HD patients to other vaccines, there are concerns regarding the robustness and durability of the humoral response induced by SARS-CoV-2 vaccines in this population.

All patients undergoing HD (about 12,000 in Portugal) received 2 doses of the Pfizer-BioNTech mRNA BNT162b2 vaccine 3 weeks apart, according to the manufacturer's and health authority's recommendations, in January–February 2021. The third dose of vaccination for elderly people, including dialysis patients, was approved in October 2021, a date posterior to the present study. Other SARS-CoV-2 vaccines distributed in Portugal (Moderna mRNA-1273, the vectorial Oxford/AstraZeneca-AZD1222, and Janssen-Ad26.COV2.S) were not administrated to HD patients.

In the general population, as evidenced in the 2–3 months follow-up of large-scale cohorts of reference health care workers (HCW), the 2-dose regimen of BNT162b2 is highly immunogenic and confers robust protection to COVID-19 and SARS-CoV-2 infection (11–13). In HD patients, initial studies revealed success in antibody generation, but reduced titers in comparison with healthy controls (14–16). Assessing the effectiveness of BNT162b2 in reducing infection, transmission, and severe disease requires very large cohorts, which for HD patients would require multicenter analysis. Hence, for SARS-CoV-2 as for other vaccines (above), antibodies could be used as proxy/biomarkers of vaccine immunity.

In this study, we aimed to evaluate the immunogenicity of mRNA BTN162b2 during the induction phase, the persistence, and decline of specific antibodies up to 6 months after initiation of the vaccination, and factors predicting these outcomes in patients undergoing HD.

MATERIALS AND METHODS

Ethics Statement

This study was reviewed and approved by the Ethics committees of DaVita in Portugal (date 2021/03/06), Centro Hospitalar Lisboa Ocidental (Reference 2102, date 2021/01/12), and the Administração Regional de Saude Lisboa e Vale do Tejo (Reference 2105/CES/2021-date 2021/03/22) in compliance with the 1975 Declaration of Helsinki, as revised in 2013, and follows the international and national guidelines for health data protection. All participants provided their written informed consent to participate in the study.

Study Design

Patients were recruited using a non-probabilistic method by convenience and volunteer sampling. The study design was planned for a universe of 170 patients, based on the number of outpatients at the participating HD center. The study enrolled 156 patients with stage 5 chronic kidney disease (CKD) undergoing renal replacement therapy as outpatients at a single HD clinic (DaVita, Eurodial) in Óbidos, Portugal. An age-matched control cohort, without kidney disease, comprised 143 individuals selected from a larger cohort of 1,245 HCW and 146 nursing home residents (17). The effect size was calculated based on the Cohen's h method to establish the power analysis, which denoted that to detect a difference of 25% with significance level of $p < 0.05$ and power analysis of 80%, we need around $n = 50$ in each group. The group ≤70 years and >70 years are $n = 66$ and $n = 77$, respectively. Stratification by age range (27–70) years and (71–93) years, splits both patient and control cohorts equally in $n = 66$ and $n = 77$ participants, in the respective age category. All patient and control participants initiated BNT162b2 mRNA vaccination (Comirnaty®, Pfizer/BioNTech) according

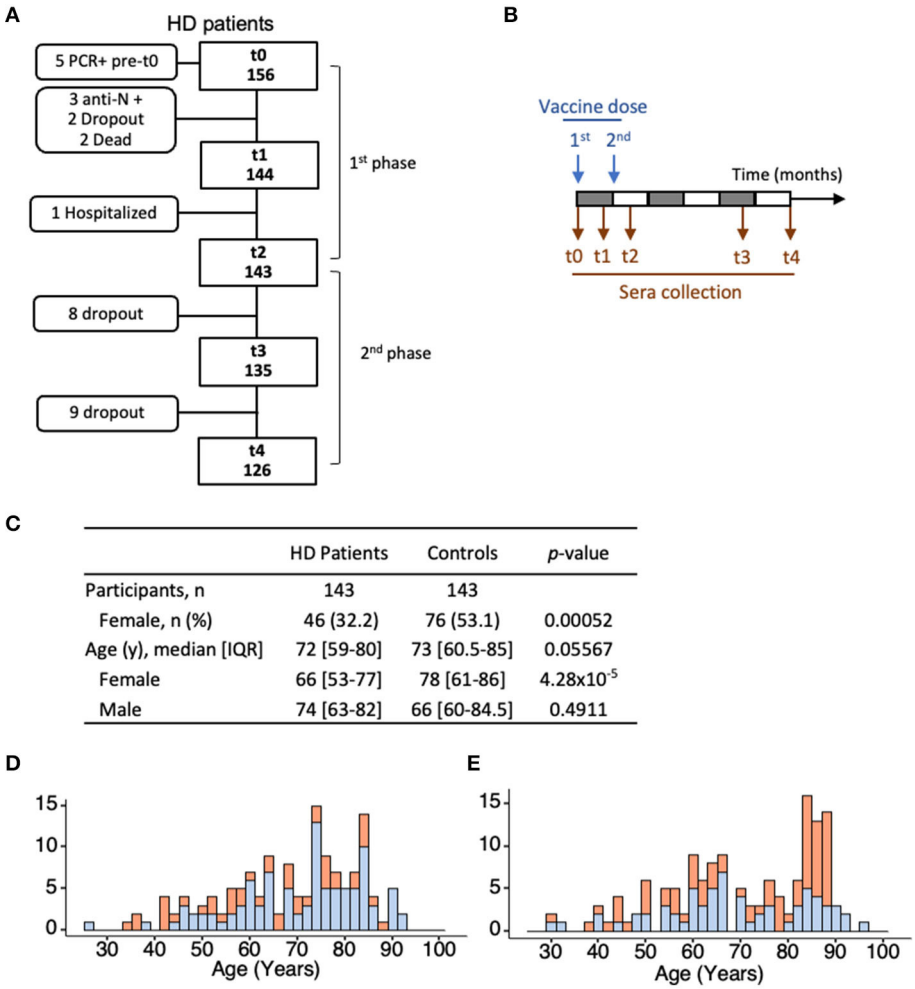


FIGURE 1 | Patient and control cohorts. **(A)** Enrolment and funneling of HD patients during the first and second phases of the study, showing concordance to the study design (straight boxes), and exclusion criteria to the antibody analysis, dropouts, and death (rounded boxes). **(B)** Serum collections were performed at the time of inoculation of the first dose (t0); 21 days post-first dose (t1) and 42 days post-first dose (t2), and thereafter, at t3 (140 days post-first vaccine dose) and t4 (180 days post-first vaccine dose). **(C–E)** Age and sex profiles of the 143 patients and 143 controls analyzed for the first phase of the study. Differences in age and sex distribution between the two cohorts were evaluated using the Wilcoxon rank sum test (with continuity correction) and Pearson's Chi-squared test (with Yates' continuity correction), respectively. HD, hemodialyzed patients; *n*, number of individuals with a given event; IQR, interquartile range.

to the established schedule of 2 doses with a 3-week interval. For the first phase of the study (Immunogenicity), venous blood was collected on the day of the first vaccine dose (time 0, t0), 3 weeks later on the day of the second dose (t1), and 3 weeks after the second dose (t2). Participants with evidence of COVID-19 infection were excluded [serum reactivity against SARS-CoV-2 nucleocapsid (N) at time of enrolment ($n = 3$) or SARS-CoV-2 RNA positivity in RT-PCR test before enrolment ($n = 2$) or during the collection time ($n = 3$), in the patient cohort] (**Figures 1A,B**). The same selection was applied to the control cohort (17). Between t0 and t1, two patients died, and two patients dropped-out of the study. Between t1 and t2, one patient was hospitalized with a non-COVID-19 respiratory infection. For the second phase of the study (Antibody persistence), venous blood was collected from 126 patients at 140 days (t3) and 180 days (t4) post-first vaccine dose. In all cases, blood collections were performed before HD procedures were initiated. Patients who did not contribute to the 3 collection times of each study phase were excluded from the analysis. Clinical data were collected from medical records and a dedicated questionnaire.

Antibody Measurements

The ELISA assay, used to quantify IgG, IgM, and IgA anti-full-length SARS-CoV-2 spike was adapted from (18), relies on antigen produced as in (19), was semiautomized to a 384-well-format and uses sera diluted at 1/50, according to a protocol to be detailed elsewhere. Assay performance was determined by testing 1,000 prepandemic sera and 40 COVID-19 patients diagnosed at least 10 days prior to sera collection. ROC curve analysis was determined at a specificity of 99.3, 99.2, and 99.2%,

TABLE 1 | Clinical characterization of HD patients classified as non-responders or responders according to anti-spike IgG levels at t2.

Characteristics	Non-responders (*N* = 13)	Responders (*N* = 130)
Sex, men, *n* (%)	9 (69.2)	88 (67.7)
Age (years), median [IQR]	86 [74–90]*	71 [59–79]*
Body Weight (Kg), median [IQR]	69 [56–74]	72 [63–83]
BMI (kg/m^2), median [IQR]	24.7 [21.9–25.7]	26 [23–30]
Dialysis duration (months), median [IQR]	46 [30–116]	46 [20–113]
Kt/v, median [IQR]	1.8 [1.7–2.0]	1.7 [1.5–1.9]
Laboratory parameters		
Hemoglobin (g/dL), median [IQR]	11.7 [11.1–12.7]	11.1 [10.4–11.8]
Serum albumin (g/dL), median [IQR]	4.0[3.6–4.1]	4.0 [3.8–4.3]
Ferritin (ng/mL), median [IQR]	348 [238–520]	368 [230–527]
nPCR (g/kg/day), median [IQR]	0.94 [0.90–1.23]	1.11 [0.95–1.22]
CRP (mg/dL), median [IQR]	0.55 [0.20–2.81]	0.48 [0.15–1.25]
25(OH)D3 (ng/mL), median [IQR]	35.0 [29.9–48.6]	35.3 [26.0–45.0]
Comorbidities		
Age adjusted Charlson score, median [IQR]	8.0 [6.0–9.0]	7.0 [5.0–8.7]
Diabetes mellitus, *n* (%)	7 (53.8)	64 (49.2)
Cardiac disease (except essential hypertension) *n* (%)	7 (53.8)	55 (42.3)
Essential hypertension, *n* (%)	8 (61.5)	96 (73.8)
Congenital or acquired immunodeficiency, *n* (%)	–	6 (4.6)
Chronic pulmonary disease, *n* (%)	–	17 (13.1)
Chronic liver disease, *n* (%)	1 (7.7)	6 (4.6)
Rheumatic disease, *n* (%)	2 (15.4)	6 (4.6)
Cancer in the last 5 years (non-leukemia), *n* (%)	1 (7.7)	13 (10)
Tumor metastasis, *n* (%)	–	2 (1.5)
Leukemia, *n* (%)	2 (15.4)†	2 (1.5)†
Past Kidney transplant, *n* (%)	3 (23.1)	20 (15.4)
Kidney allograft still present, *n* (%)	3 (23.1)‡	7 (5.4)‡
Medication		
Erythropoiesis-stimulating agent, *n* (%)	8 (61.5)	101 (77.7)
Angiotensin-converting-enzyme inhibitor, *n* (%)	1 (7.7)	29 (22.3)
Statins, *n* (%)	6 (46.2)	66 (50.8)
Corticosteroid (Prednisolone 2.5–5mg/day), *n* (%)	3 (23.1)	8 (6.2)
Other immunossupressor/immunomodulator, *n* (%)	2 (15.4)	3 (2.3)
Tacrolimus, *n* (%)	1 (7.7)	2 (1.5)
Tacrolimus and Everolimus, *n* (%)	1 (7.7)	–
Hydroxychloroquine, *n* (%)	–	1 (0.8)
Non-steroidal anti-inflammatory drug, *n* (%)	2 (15.4)	9 (6.9)
Antithrombotic, *n* (%)	8 (61.5)	70 (53.8)
Antiviral, total *n* (%)	1 (7.7)	2 (1.5)
Aciclovir, *n* (%)	1 (7.7)	–
Abacavir, Lamivudine, Efavirenz, *n* (%)	–	1 (0.8)
Abacavir, Lamivudine, Raltegravir, *n* (%)	–	1 (0.8)
Ongoing Chemotherapy, *n* (%)	–	1 (0.8)
Anti-HBc positivity, *n* (%)	1 (7.7)	13 (10)
Anti-HBs positivity (>10 UI/L), *n* (%)	5 (38.5)	62 (47.7)
Anti-HBs positivity in anti-HBc negative, *n* (%)	4 (30.8)	50 (38.5)

N, total number of individuals; n, number of individuals with a given event; t2, sera collected 42 days post-first vaccine dose; %, percentage; IQR, interquartile range; BMI, Body Mass Index; KT/V, measure of dialysis adequacy; nPCR, Normalized Protein Catabolic Rate; CRP, C-reactive protein; 25(OH)D3, calcifediol or vitamin D hydroxylated at the 25 Carbon; ESA, Erythropoiesis-stimulating agents; Anti-HBc, Hepatitis B core antigen antibodies; Anti-HBs, Hepatitis B surface antigen antibodies. Statistical tests to compare non-responders with responders were applied according to the type of variable (categorical—Fisher's exact test or continuous—Wilcoxon rank sum test).

**W = 1,200, p-value = 0.0128; 95% CI [3.00–18.00]; †p-value = 0.0414; ‡p-value = 0.0488; All others, not significant.*

and a sensitivity of 95.9, 61.2, and 73.7% for IgG, IgM, and IgA, respectively. Individual assay readouts (OD values) were standardized using calibrators (pool of positive sera at predefined dilutions) and the normalized OD (ODnorm), adjusted to set ODnorm = 1 as the positivity cut-off for IgG, IgM, and IgA. Serial titration of 67 COVID-19 patients established that the assay is semiquantitative, has a dynamic range of 3 log titer, and with decreased discrimination power at ODnorm $\geq$ 1.8. Each sample was assayed in duplicates and any identified discrepancies were resolved by repeating the test. Antibodies against SARS-CoV-2 N antigen were measured by an electrochemiluminescence immunoassay from Roche Diagnostics (Elecsys® Anti-SARS-CoV-2). Total IgG, IgM, and IgA at t2 were quantified using three immunoturbidimetric methods (PEG enhanced) from Siemens Healthineers, using Siemens Atellica CH Analyzer, following manufacturer's instructions.

Statistical Analysis

Quade test was used to analyze individuals in temporal series, and Wilcoxon signed-rank test to analyze pairwise group comparisons between different time points, which includes the Benjamini-Hochberg (BH) method for *p*-value adjustment. The Wilcoxon rank sum test (Mann–Whitney *U*-Test) was used for pairwise comparison between age groups or for single time-point comparisons between control and patient groups. To test for the effect of clinical conditions, within a given group, on the magnitude of the antibody responses, the Wilcoxon rank sum test was used. All *p*-values in multiple comparisons were adjusted using the Benjamini-Hochberg (BH) method. Pearson's Chi-squared test (with Yates' continuity correction) was used to determine differences in Ig positivity between groups over time, and within groups at specific time points. Fisher's exact test was used to test for the effect of specific clinical parameters or treatments with categorical variables on Ig positivity when assumptions for the chi-squared test were not met. Correlation of Ig levels with clinical parameters was tested by linear regression using the Spearman correlation coefficient (ρ). All *p*-values were obtained with two-sided tests, at a significance level of 0.05. All statistical tests were carried out using established R scripts. For data management, graphical design, and statistical analysis we used R, version 4.0.4 GUI 1.74 and Rstudio version 1.1.463, and the main packages tidyverse, ggplot2, openxlsx, writexl, officer, rvg, and ggpubr (references in **Supplementary Material**). The text reports continuous variables as medians and interquartile ranges (IQR), and categorical variables are summarized using frequencies and percentages.

Missing Data Management

Anti-spike antibodies measurements were performed on all participants who adhered to the study design (no missing data). For correlation analysis effects of clinical conditions, clinical parameters, or biometrics, whenever there were participants with variables not recorded, they were excluded from the analysis, and "*n*" is indicated in each figure and table.

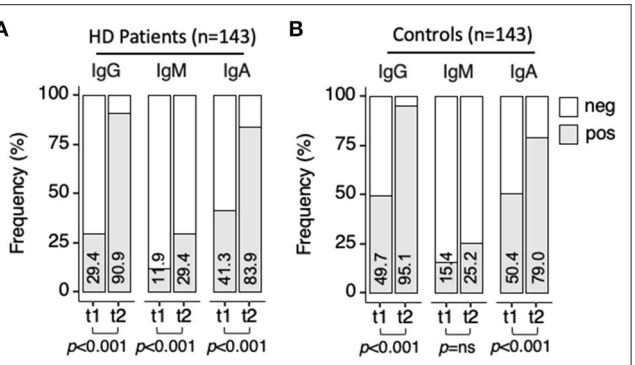


FIGURE 2 | Anti-SARS-CoV-2-spike seroconversion upon vaccination. Sera collected at t1 and t2 were analyzed for anti-spike IgG, IgM, and IgA antibodies by ELISA. ODnorm$\geq$1 was used as cut-off for positivity. Frequency of samples testing positive (gray bar) at t1 or t2 for each antibody class. **(A)** HD patients (n = 143). **(B)** controls (n = 143). Percentage of seroconversion indicated inside each bar. Pearson's Chi-squared test (with Yates' continuity correction) was used to determine differences over time in patients and controls (*p*-values indicated in the figure) and between patients and controls at t1, and t2 (*p*-values indicated in **Supplementary Tables 1, 2**). neg, negative; pos, positive; t1, sera collected 21 days post-first vaccine dose; t2, sera collected 42 days post-first vaccine dose.

RESULTS

Cohort Characterization

This longitudinal prospective cohort study enrolled 156 patients on HD scheduled for BNT162b2 mRNA vaccination in January and February 2021 (**Figure 1**). A total of 143 participants, with no evidence of previous exposure to SARS-CoV-2 at the first collection (anti-N-antigen negative, no previous PCR tests positive), adhered to the three collection times of the first phase of the study addressing the vaccine immunogenicity. The median age was 72 years of age (y) [range (27–93), IQR (59–80)], and women represented 32% of the cohort. Eleven patients (8.8%) were under therapies potentially affecting immune responses, including corticosteroids (**Table 1**). The control cohort included 143 individuals with median age of 73 y [range (30–96), IQR (61–85)] and 53% women. For the second phase of the study addressing antibody persistence, 126/143 patients adhered to the additional two collection times.

Antispike Antibody Response in the Induction Phase

Sera from HD patients and controls were analyzed for specific anti-SARS-CoV-2-spike antibodies (IgG, IgM, and IgA) using an ELISA calibrated with sera collected prior COVID-19 pandemic and from COVID-19 patients. We first analyzed seroconversion, discriminating positive/negative antibody reactivity (**Figure 2**, **Supplementary Tables 1, 2**). At t0, before vaccination, 100% of the control and 141/143 of the HD patients tested negative for anti-spike Ig. After a single vaccine dose (t1), seroconversion was lower in HD patients with only 42/143 (29.4%; 95%CI 22.5–37.3) patients developing anti-spike IgG antibodies when compared with 71/143 (49.7%; 95%CI 41.6–57.7) controls (patients vs.

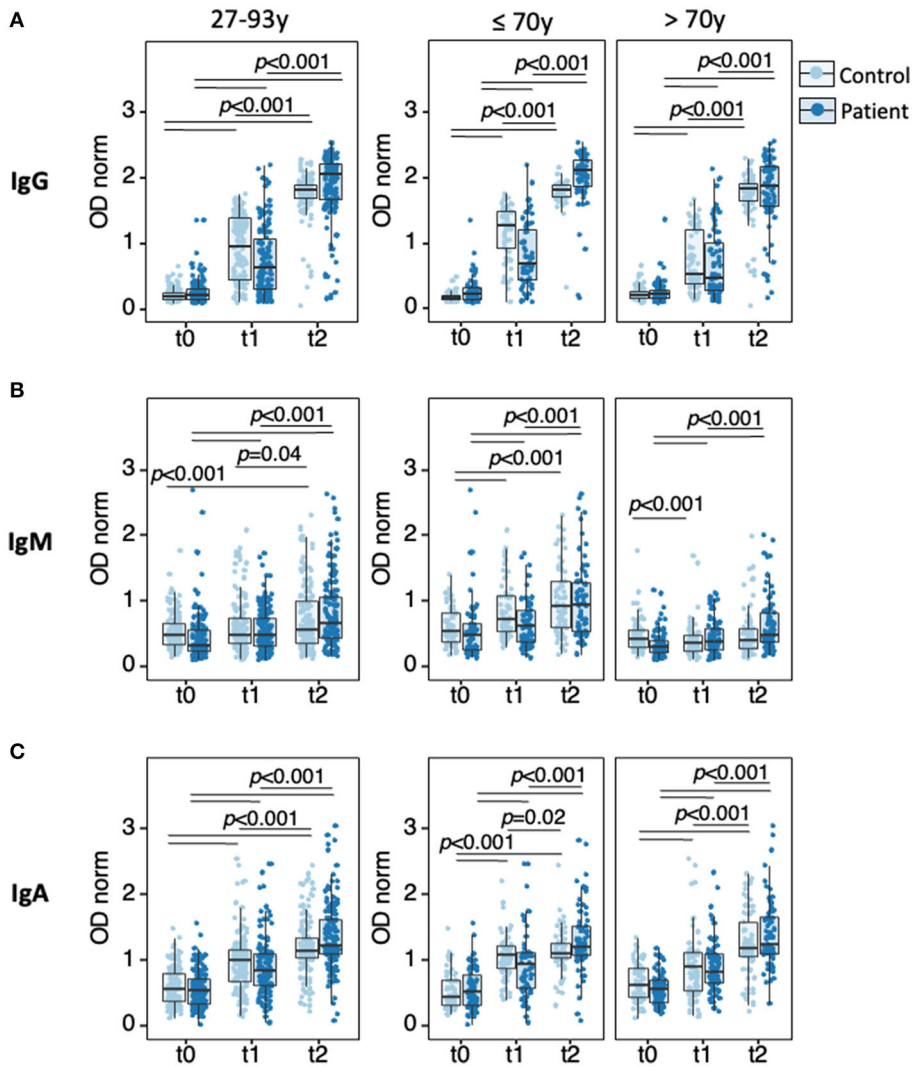


FIGURE 3 | Anti-SARS-CoV-2-spike IgG, IgM, and IgA responses to vaccination. Sera collected at t0, t1, and t2 were analyzed by ELISA for semi-quantitative measurement of anti-spike IgG **(A)**, IgM **(B)**, and IgA **(C)** in HD patients (dark blue, $n = 143$) and age-matched controls (light blue, $n = 143$), in the full cohort (left panels) or upon stratification by age group (≤70 y, middle panels; >70 y, right panels). Data points represent individual subjects and are overlaid with boxes representing interquartile range (IQR), whiskers representing 1.5 IQR tails, and a central line representing median value. Differences were determined by Quade test for antibody levels along time in the full cohort (all 9 panels), Wilcoxon signed-rank test for pairwise comparison in each panel (*p*-values indicated by horizontal black bars), and Wilcoxon rank-sum to compare controls with patients at t1. Detailed *p*-values presented in **Supplementary Tables 3–7**. t0, sera collected on the day of first vaccine dose; t1, sera collected 21 days post-first vaccine dose; t2, sera collected 42 days post-first vaccine dose.

controls at t1, $p = 0.0014$). The second vaccine dose acted as a boost in both cohorts (t1 vs. t2, patients $p = 8.01 \times 10^{-26}$, controls $p = 2.59 \times 10^{-17}$), and both cohorts reached similar seropositivity rate (HD patients 130/143, 90.9%, 95% CI 85.1–94.6 and controls 136/143, 95.1%, 95%CI 90.2–97.6, $p = 0.2463$). Isotype class analysis of anti-spike antibodies revealed progression of IgA seroconversion in HD patients from t1 to t2 (41.3%; 95%CI 33.5–49.5 at t1, 83.9%; 95%CI 77.0–89.0 at t2, $p = 2.27 \times 10^{-13}$), reaching values similar to the control cohort ($p = 0.3612$). In contrast, prevalence of anti-spike IgM was low with modest increase along the vaccination schedule in both patients (11.9%; 95%CI 7.6–18.2 at t1 and 29.4%; 95%CI 22.5–37.3 at t2, $p = 0.0005$) and controls (15.4%, 95%CI 10.4–22.2 at t1 and 25.2%, 95%CI 18.8–32.9 at t2, $p = 0.0559$).

Semiquantitative analysis of antibody levels using normalized OD values (ODnorm) revealed significant increase of all three isotypes from t0 to t1, an effect of the first vaccine dose, and from t1 to t2, an effect of the second vaccine dose, in both patients and controls (**Figure 3**, **Supplementary Tables 3–7**). Within the patient cohort, most striking was the boosting effect of the second dose on anti-spike IgG levels (median [IQR]: 0.63 [0.32–1.08]) at t1; 2.05 [1.67–2.23] at t2, $p = 2.2 \times 10^{-16}$) and to a lower extent on anti-spike IgA levels (median [IQR]: (0.85 [0.63–1.10] at t1; 1.22 [1.10–1.63] at t2, $p = 2.2 \times 10^{-16}$), while anti-spike IgM was

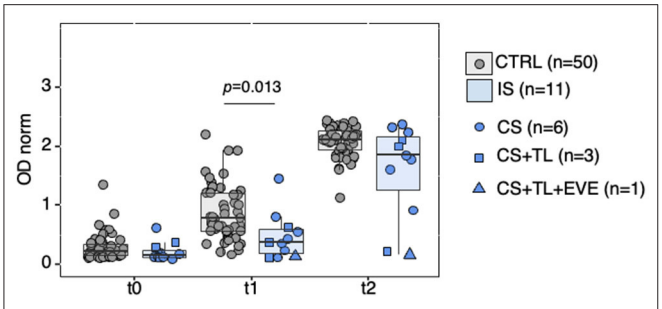


FIGURE 4 | Anti-SARS-CoV-2-spike IgG reactivity in HD patients under immunosuppressive therapy. Partitioning of data presented in **Figure 3**, showing HD patients treated with immunosuppressive drugs (IS, $n = 11$) or not (CTRL, $n = 50$). Data points represent individual subjects and are overlaid with boxes representing interquartile range (IQR), whiskers representing 1.5 IQR tails, and a central line representing median value. At each time point differences in antibody levels between groups were determined with the Wilcoxon rank sum test (with continuity correction); significant *p*-values indicated in the figure. IS, immunosuppressive drugs; CTRL, controls; CS, corticosteroids; TL, Tacrolimus; EVE, Everolimus. Detailed *p*-values presented in **Supplementary Table 9**. t0, sera collected on the day of first vaccine dose; t1, sera collected 21 days post-first vaccine dose; t2, sera collected 42 days post-first vaccine dose.

only modestly increased (0.49 [0.32–0.75] at t1; 0.66 [0.45–1.07] at t2, $p = 4.5 \times 10^{-15}$).

Comparison between patients and controls revealed lower anti-spike IgG levels in patients after the first vaccine dose (0.63 [0.32–1.08] in patients; 0.96 [0.46–1.39] in controls, $p = 4.98 \times 10^{-04}$), an effect not observed for the other isotypes.

To test whether the lower response of HD patients to the first vaccine dose holds across age, each cohort was partitioned in two age groups (right panels in **Figure 3**), $\leq$ 70 y (range (27–70), median 58, IQR [50–64), $n = 66$ for patients and range (30–70), median 60, IQR [51–64], $n = 66$ for controls) and >70 y (range (71–93), median 8, IQR [75–84], $n = 77$ for patients and range (71–96), median 85 IQR [70–88], $n = 77$ for controls). In controls and patients, elderly individuals presented similar low anti-spike IgG levels at t1, (0.47 [0.28–1.00] in patients; 0.52 [0.38–1.21] in controls, $p = 0.2492$). In contrast, in the $\leq$ 70 y groups, HD patients presented lower IgG response at t1 (0.68 [0.45–1.21] in patients, 1.27 [0.93–1.49] in controls, $p = 2.01 \times 10^{-05}$). In controls, age was clearly associated with lower IgG levels at t1 ($p = 5.09 \times 10^{-07}$), whereas in patients age effect was barely significant ($p = 0.050$). Together, these data indicate that the younger group contributed to most of the lower response observed at t1 when analyzing the full HD cohort. Similar analysis after the second vaccination dose could not be directly processed, as most samples in the control group reached values above the dynamic range of the assay.

Factors Predicting Immunogenicity of BNT162B2 in HD Patients

The HD cohort encompassed 11 patients under immunosuppressive (IS) therapy (age range (42–63), median 56, IQR [51.0–58.0]). All IS patients received Prednisolone 2.5–5 mg/day, a mild IS regimen. Four patients were in addition treated with Tacrolimus, a strong IS drug (**Table 1**). Levels of anti-spike IgG antibodies in this small subgroup were compared with those of a subset of patients not on IS and selected from the full HD cohort (age range (42–69), median 60, IQR [53.0–64.0], $n = 50$) **Figure 4**, **Supplementary Tables 8, 9**). The levels of anti-spike IgG antibodies elicited by the first vaccine dose (t1), but not by the second dose, were lower in IS than control patients ($p = 0.013$ at t1). Of the 4 patients under Tacrolimus therapy, 2 were non-responders and 2 were responders at t2.

Responsiveness to Hepatitis B vaccination is an indicator of immune competence. Anti-HBs antibody levels were available for 129 HD patients who tested negative for anti-HBc (hence, presumed not previously exposed to Hepatitis B virus). Of these, only 54/129 (42%) tested anti-HBs positive, whereas 117/129 (91%) were positive for anti-spike IgG at t2. Responsiveness to Hepatitis B and to BNT162b2 vaccine at t2 did not correlate, either measuring seroconversion ($p = 0.53$; OR = 1.49, 95% CI [0.48–4.65]) (**Figure 5A**) or specific antibodies levels ($\rho = 0.062$, $p = 0.48$) (**Figure 5B**). Likewise, neither total IgG levels nor lymphocyte counts measured for 142 patients at t2, correlated with anti-spike IgG levels (**Figure 5C**, **Supplementary Figure 1**).

We next analyzed indicators of kidney disease severity or activity. Neither time in dialysis nor levels of 25-hydroxycholecalciferol, C-reactive protein, hemoglobin, Ferritin, epoetin dosage, nor normalized protein catabolic rate significantly correlated with anti-spike IgG levels (**Supplementary Figure 1**).

Reviewing the clinical data of the 13/143 HD patients who remained anti-spike IgG negative at t2 was not informative due to the small sample size in each clinical category, and possible confounding factors due to multiple comorbidities (**Table 1**). Among these 13 non-responders, 3 were in their 50th, 3 in their 70th decade, and 7 were >84 y. In the control cohort, 7 participants were non-responders, 1 was 54 and under strong IS therapy, and 6 were >84 y. Altogether, these results support advanced age, and incidentally, immunosuppression is a *bona fide* factor affecting seroconversion in the general population, as for HD patients. Finally, and in concordance with multiple factors conditioning the amplitude of the humoral response to BNT162b2 vaccine, the age-adjusted Charlson comorbidity index was weakly inversely correlated with anti-spike IgG levels at t2 ($\rho = -0.3$, *p*-value = 0.0003) (**Figure 5D**).

Persistence vs. Seroreversion of Antispike Responses

Waning of the humoral response in HD patients was assessed in 126/143 patients who complied with two additional collections at t3 (140 days after first vaccine dose, corresponding to 4 months post second dose) and t4 (180 days after first vaccine dose, corresponding to 5 months post second dose) (**Figure 6**, **Supplementary Table 10**). Anti-spike IgG levels decreased in the 100-day interval between t2 and t3 and during the following 40 days between t3 and t4 (median [IQR]: 2.03 [1.69–2.21] at t2; 1.49 [1.08–1.79] at t3; 1.28 [0.84–1.58] at t4, t2 vs. t3 and t3 vs. t4 $p < 2 \times 10^{-16}$). IgM and IgA antibodies levels, which were

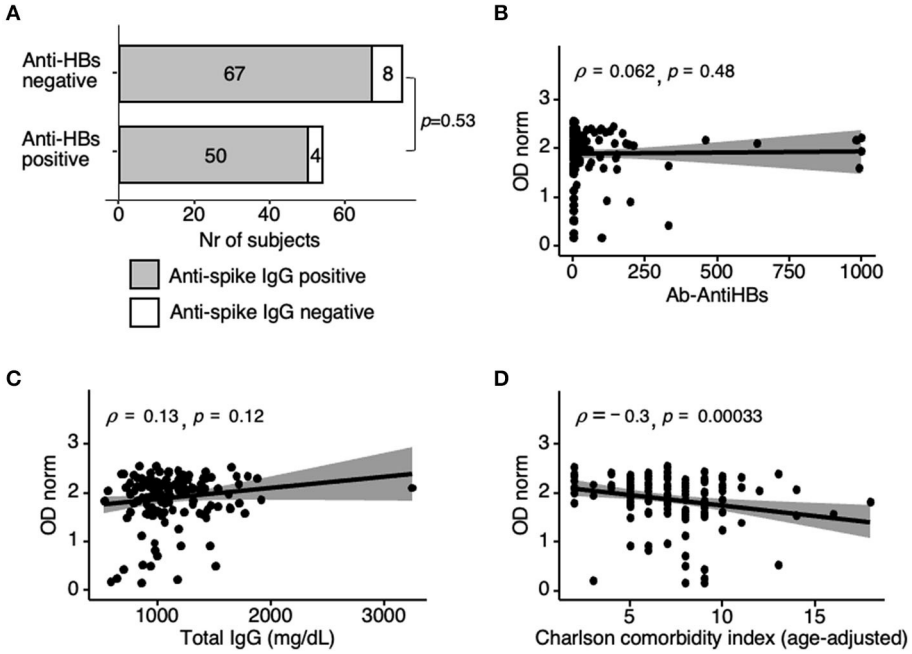


FIGURE 5 | Correlation analysis of anti-spike IgG responses at t2. **(A)** Anti-spike positivity as in **Figure 2**, now in $n = 75$ non-responders and $n = 54$ responders to previous hepatitis B vaccination (anti-HBs antibody cut-off >10 mIU/ml). Fischer's test p-value = 0.53; OR = 1.49, 95%CI [0.48–4.65]. Fourteen anti-HBc reactive participants (i.e., previously infected with HBV) were excluded. **(B)** Spearman correlation coefficients (ρ) analysis of anti-spike IgG with anti-HBs antibody levels in anti HBc non-reactive individuals ($n = 129$, same as in **A**). **(C,D)** Spearman correlation coefficients (ρ) analysis of anti-spike IgG levels with total serum IgG also determined at t2 ($n = 142$) **(C)**, and with age-adjusted Charlson-comorbidity index ($n = 142$) **(D)**. Shaded areas represent 95% confidence interval. Differences were determined by Spearman's rank-order non-parametric test; t2, sera collected 42 days post-first vaccine dose.

not elevated at t2, decreased between t2 and t3 ($p < 2 \times 10^{-16}$ for both) and remained at the same low values between t3 and t4 ($p = 0.750$ for IgM and $p = 0.410$ for IgA), confirming IgG is the dominant class of reactive antibodies elicited by BNT162b2 vaccine. Anti-spike IgG waning over time led to a progressive decrease in positivity, resulting in 39/126 (31%) seronegatives at t4.

Of the 39 patients who presented values below the threshold of positivity by t4, 10 were originally non-responders whereas 29 (23% of the cohort) were *bona-fide* seroreverters (**Table 2**). As for anti-spike seroconversion, anti-spike seroreversion did not correlate with HBV vaccination response, with 11/29 (37.9%) seroreverters and 44/87 (50.6%) seropositive at t4 presenting anti-HBs antibodies ($p = 0.286$). Analysis of anti-spike IgG levels along the 5 time points (**Figure 7**, **Supplementary Tables 11–13**) revealed that patients who remained seropositive 5 months post second dose (t4, $n = 87$) presented higher anti-spike IgG levels at earlier time points. This was already evident as early as 3 weeks after the first dose (t1) (0.80 [0.44–1.22] in positive vs. 0.37 [0.24–0.70] in the negative, $p = 7.03 \times 10^{-04}$). Analysis performed on the 116 participants that either maintained or lost IgG positivity at t4 confirmed IgG levels at earlier time points, including t1, correlated with values at t4 ($\rho = 0.58$, $p < 5.6 \times 10^{-12}$ for t4 vs. t1). In agreement with the latter finding, and as for t2, age barely contributed to seroreversion at t4 ($p = 0.027$), and no specific clinical conditions or treatment could explain this outcome (**Table 2**).

DISCUSSION

In this study, we evidence that while seroconversion following priming was lower in HD patients when compared to age-matched controls, most reach positivity for anti-spike IgG after a second BNT162b2 vaccine dose. Furthermore, waning of the humoral immune response is readily detectable 4 months after the second vaccination. Together, these findings further advocate for the specific management of HD patients during the COVID-19 pandemic.

The original guidelines for BNT162b2 vaccine regimen were of 2 doses administrated at 3–4 weeks interval, although the time between doses has been debated, and more recently, a reinforcing third dose has been approved for specific population subgroups. Our data confirmed that the second dose is essential to reach a high frequency of seroconversion in HD patients, as was shown before in smaller cohorts [e.g., (20), $n = 22$, (21), $n = 10$]. The heterogeneity we evidence in the levels of reactive Ig after a single vaccine dose, with close to 70% seronegative patients, advocates not to extend the interval between the 2 doses to rapidly reach high levels of antibodies. This proposition

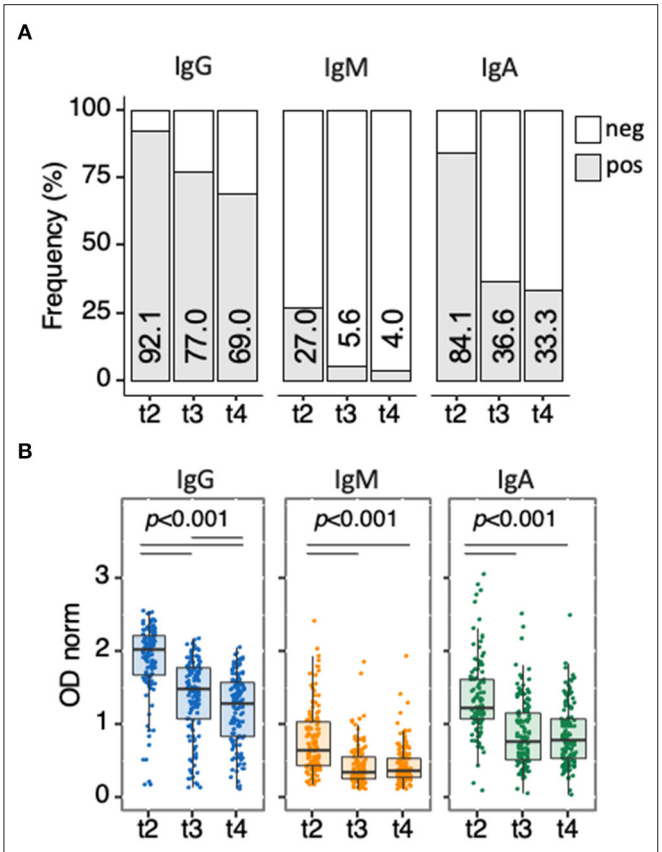


FIGURE 6 | Waning of anti-spike antibody responses along time. Follow-up analysis of antibody responses for anti-spike IgG, IgM, and IgA antibodies in HD patients ($n = 126$) collected at t2, t3, and t4. **(A)** Seroconversion is determined and presented as in **Figure 2**. Pearson's Chi-squared test (with Yates' continuity correction) was used to determine differences over time. **(B)** anti-spike antibodies in patients, determined and presented as in **Figure 3**. Differences were determined by the Quade test for antibody levels along time (all 3 panels), the Wilcoxon signed-rank test for pairwise comparison in each panel (*p*-values indicated by horizontal black bars). Detailed *p*-values presented in **Supplementary Table 10**. t2, sera collected 42 days post-first vaccine dose; t3, sera collected ∼140 days post-first vaccine dose; t4, sera collected 180 days post-first vaccine dose.

is consistent with a previous study addressing a very large population which evidenced BNT162b2 vaccine effectiveness was of 57% after one dose and 97% after two doses (11). Other studies argue that extending the time of prime-boost interval enhances the recall response (22), which may be beneficial in the long term, notably by prolonging immune memory. However, in times of pandemic, with actual or risk of high infection incidence, frail populations would benefit from rapidly reaching effective immunity.

In our immunogenicity analysis, we excluded participants who were identified as previously exposed to SARS-CoV-2, so as to evaluate the immune response induced *de novo* by the vaccine. In preexposed individuals, the first vaccine dose acts as a boost (23–26), as confirmed in our enlarged cohort of controls after analyzing specifically the anti-N positive participants at t0 (17), who were excluded in this work. However, some participants may have been preexposed to SARS-CoV-2 and already lost anti-N reactivity by the time the study was initiated (27). It is plausible that the 2 HD participants found anti-spike IgG positive at t0 were preexposed and had already lost anti-N reactivities by the time of our analysis.

One strength of our study is the partitioning of the patient and control cohort by age groups. With this approach, we reveal the difference between HD patients and controls lies in the younger participants. This finding is relevant as a third booster shot has just been approved and has been prioritized by age and specific conditions that do not include HD so far. Analysis upon age partitioning also completes previous studies reporting that COVID-19 vaccines are less efficacious at inducing antibody in HD patients (14, 20, 28). Nevertheless, our analysis confirms aging is a dominant trait affecting mRNA vaccine effectiveness (17, 29). Finally, our finding that the younger group of patients shows lower levels of anti-spike reactivities than age-matched control, and is barely differentiated from aged patients, is compatible with a signature of early immune senescence in this population.

Although IgG is the dominant class of anti-spike antibodies induced upon BNT162b2 vaccination, our results indicate 84% of the patients mounted an IgA response. Secretory IgA, the product of *a bona fide* germinal center reaction, acts at the mucosa, the site of primary SARS-CoV-2 infection, and anti-spike secretory IgA responses with neutralizing capacity were reported following natural SARS-CoV-2 infection (30). Whether the vaccine reactive IgA encompasses secretory IgA remains to be assessed. The IgM levels were relatively low in both cohorts, possibly related to IgM being produced transiently as the result of a rather T-cell independent process. In support of a rather T-cell independent response for both IgA and IgM, the decline in reactivity for these isotypes was severe by t3 in most patients.

Only 42% of patients in the HD cohort presented anti-HBs antibodies following Hepatitis B vaccination. A similar range of hepatitis B vaccination effectiveness was reported previously (31). The lack of correlation between anti-spike Ig levels, or seroconversion, after BNT162b2 vaccination at t2 as at t4 and responsiveness to hepatitis B immunization is in accordance with a previous publication addressing a smaller cohort ($n = 81$) at early time point post SARS-CoV2 vaccination (32). The Hepatitis B vaccine is a subunit vaccine (HBs antigen mixed with adjuvant), while BNT162b2 is an mRNA embedded in lipid nanoparticles. The findings may support that mRNA vaccines present increased immunogenicity when compared with more standard subunit vaccines. Alternatively, spike may be more immunogenic than HBs. Irrespectively of these considerations, serial recall injections are common practice for Hepatitis B vaccine in identified antibody negative individuals. Similarly, serial recall BNT162b2 vaccinations may be required for those individuals identified as poor responders.

After a prime-boost induction phase, vaccine-reactive antibody levels are expected to decrease. However, for most

TABLE 2 | Clinical characterization of nonresponders, responders who lost (seroreverted), and responders who maintained (seropositive) anti-spike IgG at t4.

Characteristics	Sero-reverted (*N* = 29)	Seropositive (*N* = 87)
Age (years), median [IQR]	76 [64–84]*	69 [59–79]*
Sex, men, *n* (%)	19 (65.52)	61 (70.11)
Dialysis duration (months), median [IQR]	46 [23–99]	47 [20–121]
Comorbidities		
Charlson Score (age–adjusted), median [IQR]	8 [6–9]	7 [5–8]
Obesity (BMI>30 Kg/m^2), *n* (%)	11 (37.93)	23 (26.44)
Endocrine diseases (Diabetes mellitus and others), *n* (%)	17 (58.62)	41 (47.13)
Cardiovascular disease, excluding essential hypertension, *n* (%)	16 (55.17)	33 (37.93)
Essential hypertension, *n* (%)	23 (79.31)	64 (73.56)
Congenital or acquired immunodeficiency, *n* (%)	3 (10.34)	3 (3.45)
Chronic pulmonary disease, *n* (%)	8 (27.59)	10 (11.49)
Chronic liver disease, *n* (%)	0	6 (6.90)
Rheumatic disease, *n* (%)	0	5 (5.75)
Cancer in the last 5 years (non-leukemia), *n* (%)	4 (13.79)	16 (18.39)
Past Kidney transplant, *n* (%)	6 (20.69)	13 (14.94)
On immunosuppressive drugs, *n* (%)	1 (3.45)	6 (6.90)

N, total number of individuals; n, number of individuals with a given event; %, percentage; IQR, interquartile range; t4, sera collected 180 days post-first vaccine dose.
Statistical tests to compare sero-reverters with seropositives at t4 were applied according to the type of variable (categorical - Fisher's exact test or continuous—Wilcoxon rank sum test).
**W = 2,042, p-value = 0.0273, 95% CI [1.00–11.00].*
All others, not significant.

vaccines, long-term immunological memory is associated with detectable antibody reactivities in healthy individuals, albeit at low levels. This is the case for BNT162b2, as 6 months post-second dose a vast majority of 1,370 HCW cohort were still seropositive (33). However, waning vaccine-induced immunity in the general population by around month 4 postvaccination is revealed in countries with high SARS-CoV-2 incidence, a discrepancy likely related to the change in the dominant variant of SARS-CoV-2, which partially escapes immunity induced by the ancestral form of the spike (34). In HD patients, longitudinal studies addressing decreased immunity upon mRNA vaccination are still scarce. It was recently reported that 10/172 HD patients (6%) seroревert by 3 months after the second vaccine dose (35). Another study conducted on 41 HD patients, indicates that seroconversion rate decreases from 98% at 1 month to 66% at 6 months after the second dose (36). In our work, we dissociated non-responders to the 2-dose vaccine regimen from *bona fide* seroreverters, studied a cohort of similar size to that in (35), and a duration approaching that of (36) to reveal 29/145 (20%) lost positivity in the 4 months following the second dose, a result in accordance with the previous studies. We evidenced that levels of specific IgG after the first or second dose can serve as predictors of the persistence of seroconversion, a correlation reported previously in a smaller ($n = 41$) cohort (36). Altogether, our kinetic analyses support the previous proposition of additional booster doses for this group of vulnerable patients (37).

We tested whether seroconversion and seroreversion were correlated with immunosuppressive therapies or with disease duration, severity, or activity, and found only signals of little significance. This result may be related to only few patients being under immunosuppressors in our cohort and most of these under mild therapies, and also to the evidence HD patients are a heterogeneous group in what concerns comorbidities. In concordance with multiple factors modulating humoral immunity, only the age-adjusted Charlson comorbidity index predicted anti-spike IgG levels at t2.

The limitations of our study include that systematic surveillance for SARS-CoV-2 infections was not performed. Larger cohorts of HD patients will be necessary to evaluate vaccine effectiveness in preventing infection, morbidities or death, and viral transmission. The study did not include functional assays such as neutralizing antibodies, which have been shown to be predictive of protection from severe disease and to a lower extent from infection (38). However, levels of anti-spike reactivity elicited by SARS-CoV-2 mRNA vaccines correlate with *in vitro* neutralization of spike-pseudoviruses and SARS-CoV-2, including variants of concern, by us and others (39, 40). Moreover, both binding and neutralizing antibodies correlate with mRNA vaccine efficacy (41, 42). We also did not address cellular immunity. Previous studies in HD patients revealed a strong correlation between anti-spike antibody detection and the frequency or the total number of specific plasmablasts and memory B cells (43) and also with specific T cell responses (21). Others failed to evidence this correlation when comparing HD and controls, with decreased humoral but not T cell responses (44).

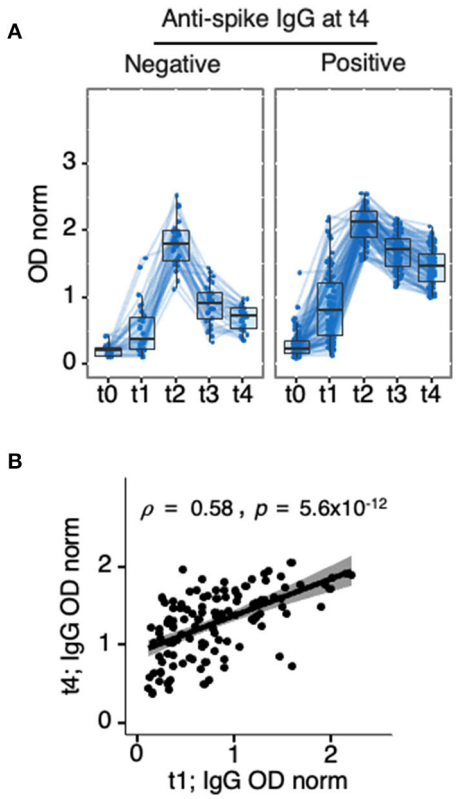


FIGURE 7 | Anti-spike IgG levels at t1 as a predictor of seroreversion. Longitudinal analysis of anti-spike IgG in HD patients who participated in the 5 collection time points (t0, t1, t2, t3, and t4). Ten non-responders (seronegative at t1 and t2) were excluded from the analysis, ($n = 116$) **(A)** Partitioning of data presented in **Figures 3**, **6**, showing HD patients classified as negative ($n = 39$, left), or positive ($n = 87$, right) at t4. **(B)** Spearman correlation coefficients (ρ) analysis of anti-spike IgG levels at t4 vs. t1 in same ($n = 126$) patients, p-value inserted in the panel was obtained by Spearman's rank-order non-parametric test. Detailed p-values presented in **Supplementary Tables 11–13**. t0, sera collected on the day of first vaccine dose; t1, sera collected 21 days post-first vaccine dose; t2, sera collected 42 days post-first vaccine dose; t3, sera collected ∼140 days post-first vaccine dose; t4, sera collected 180 days post-first vaccine dose.

Detangling such discrepancies will await further analysis and standardized protocols. Similarly to other vaccines, it remains likely that detection of reactive antibodies are positive indicators of the engagement and memory of the adaptive immune system.

Despite these limitations, our findings highlight that HD patients may benefit from tailored COVID-19 vaccination regimens and follow-up. This concern is acute as variants less susceptible to vaccine-induced immunity have replaced worldwide the ancestral virus from which BNT162b2 was derived.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committees of (i) DaVita in Portugal, (ii) Centro Hospitalar Lisboa Ocidental Portugal, and (iii) the Administração Regional de Lisboa e Vale do Tejo Portugal. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AW, JD, and CP-G conceived and designed the study, validated the data, and finalized the manuscript. M-LB performed the statistical analysis. LG and IG organized the database. ND wrote the first draft of the manuscript. PB, AB, VM, PM, OA, LK, and MM performed laboratory assays. RA, PC, EN, MP, and AF collected samples and clinical data of the test cohort. TN and JF-V organized the collection of samples and clinical data of the control cohorts. All authors approved the submitted version.

FUNDING

This work benefited from COVID-19 emergency funds 2020 from Calouste Gulbenkian Foundation and from Oeiras and Almeirim city councils. It was also supported by the Science and Technology Foundation, Ministry of Education and Science (FCT, Portugal) through Project 754-Research4COVID-19-−2nd edition, by PORLisboa 2020, Portugal 2020 and European Union, through European Regional Development Fund through the project FEDER/072558, and from research funds from DaVita, Portugal. The funding entities had no role in study design, data collection and analysis, or decision to publish.

ACKNOWLEDGMENTS

João Frazão for continuous support, fruitful discussions, and critical review of the manuscript. Ana Paula Agudo, Gabriela Teixeira, João Gomes, Jorge Araújo, and Rui Costa, for clinical data collection. Carla Brás for secretarial assistance; nursing staff of DaVita Obidos for sample collection; Affidea laboratories for sample management.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2021.796676/full#supplementary-material

REFERENCES

1. Council E-E, Group EW. Chronic kidney disease is a key risk factor for severe COVID-19: a call to action by the ERA-EDTA. *Nephrol Dial Transplant.* (2021) 36:87–94. doi: 10.1093/ndt/gfaa314
2. Carrero JJ, Stenvinkel P. Inflammation in end-stage renal disease-what have we learned in 10 years? *Semin Dial.* (2010) 23:498–509. doi: 10.1111/j.1525-139X.2010.00784.x
3. Girndt M, Sester U, Sester M, Kaul H, Köhler H. Impaired cellular immune function in patients with end-stage renal failure.

Nephrol Dial Transp. (1999) 14:2807–10. doi: 10.1093/ndt/14.12.2807
4. Eleftheriadis T, Antoniadi G, Liakopoulos V, Kartsios C, Stefanidis I. Disturbances of acquired immunity in hemodialysis patients. *Semin Dial.* (2007) 20:440–51. doi: 10.1111/j.1525-139X.2007.00283.x
5. Betjes MG. Immune cell dysfunction and inflammation in end-stage renal disease. *Nat Rev Nephrol.* (2013) 9:255–65. doi: 10.1038/nrneph.2013.44
6. Kato S, Chmielewski M, Honda H, Pecoits-Filho R, Matsuo S, Yuzawa Y, et al. Aspects of immune dysfunction in end-stage renal disease. *Clin J Am Soc Nephrol.* (2008) 3:1526–33. doi: 10.2215/CJN.00950208
7. Crespo M, Collado S, Mir M, Cao H, Barbosa F, Serra C, et al. Efficacy of influenza A H1N1/2009 vaccine in hemodialysis and kidney transplant patients. *Clin J Am Soc Nephrol.* (2011) 6:2208–14. doi: 10.2215/CJN.02160311
8. Vandecasteele SJ, De Bacquer D, Caluwe R, Ombelet S, Van Vlem B. Immunogenicity and safety of the 13-valent Pneumococcal Conjugate vaccine in 23-valent pneumococcal polysaccharide vaccine-naive and pre-immunized patients under treatment with chronic haemodialysis: a longitudinal quasi-experimental phase IV study. *Clin Microbiol Infect.* (2018) 24:65–71. doi: 10.1016/j.cmi.2017.05.016
9. Udomkarnjananun S, Takkavatakarn K, Praditpornsilpa K, Nader C, Eiam-Ong S, Jaber BL, et al. Hepatitis B virus vaccine immune response and mortality in dialysis patients: a meta-analysis. *J Nephrol.* (2020) 33:343–54. doi: 10.1007/s40620-019-00668-1
10. Vaziri ND, Pahl MV, Crum A, Norris K. Effect of uremia on structure and function of immune system. *J Ren Nutr.* (2012) 22:149–56. doi: 10.1053/j.jrn.2011.10.020
11. Haas EJ, Angulo FJ, McLaughlin JM, Anis E, Singer SR, Khan F, et al. Impact and effectiveness of mRNA BNT162b2 vaccine against SARS-CoV-2 infections and COVID-19 cases, hospitalisations, and deaths following a nationwide vaccination campaign in Israel: an observational study using national surveillance data. *Lancet.* (2021) 397:1819–29. doi: 10.1016/S0140-6736(21)00947-8
12. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N Engl J Med.* (2020) 383:2603–15. doi: 10.1056/NEJMoa2034577
13. Angel Y, Spitzer A, Henig O, Saiag E, Sprecher E, Padova H, et al. Association between vaccination with BNT162b2 and incidence of symptomatic and asymptomatic SARS-CoV-2 infections among health care workers. *JAMA.* (2021) 325:1939–40. doi: 10.1001/jama.2021.7152
14. Grupper A, Sharon N, Finn T, Cohen R, Israel M, Agbaria A, et al. Humoral response to the pfizer BNT162b2 vaccine in patients undergoing maintenance hemodialysis. *Clin J Am Soc Nephrol.* (2021) 16:1037–42. doi: 10.2215/CJN.03500321
15. Jahn M, Korth J, Dorsch O, Anastasiou OE, Sorge-Hädicke B, Tyczynski B, et al. Humoral response to SARS-CoV-2-Vaccination with BNT162b2 (Pfizer-BioNTech) in patients on hemodialysis. *Vaccines.* (2021) 9:360. doi: 10.3390/vaccines9040360
16. Lacson E, Argyropoulos CP, Manley HJ, Aweh G, Chin AI, Salman LH, et al. Immunogenicity of SARS-CoV-2 vaccine in dialysis. *medRxiv.* (2021). doi: 10.1101/2021.04.08.21254779
17. Viana JF, Bergman M-L, Gonçalves LA, Duarte N, Coutinho TP, Borges PC, et al. Population homogeneity for the antibody response to COVID-19 BNT162b2 / Comirnaty vaccine is only reached after the second dose, across all adult age ranges. *medRxiv.* (2021). doi: 10.1101/2021.03.19.21253680
18. Stadlbauer D, Amanat F, Chromikova V, Jiang K, Strohmeier S, Arunkumar GA, et al. SARS-CoV-2 seroconversion in humans: a detailed protocol for a serological assay, antigen production, and test setup. *Curr Protoc Microbiol.* (2020) 57:e100. doi: 10.1002/cpmc.100
19. Castro R, Nobre LS, Eleuterio RP, Thomaz M, Pires A, Monteiro SM, et al. Production of high-quality SARS-CoV-2 antigens: impact of bioprocess and storage on glycosylation, biophysical attributes, and ELISA serologic tests performance. *Biotechnol Bioeng.* (2021) 118:2202–19. doi: 10.1002/bit.27725
20. Speer C, Goth D, Benning L, Buylaert M, Schaier M, Grenz J, et al. Early humoral responses of hemodialysis patients after COVID-19 vaccination with BNT162b2. *Clin J Am Soc Nephrol.* (2021) 16:1073–82. doi: 10.2215/CJN.03700321
21. Bertrand D, Hamzaoui M, Lemee V, Lamulle J, Hanoy M, Laurent C, et al. Antibody and T cell response to SARS-CoV-2 messenger RNA BNT162b2 vaccine in kidney transplant recipients and hemodialysis patients. *J Am Soc Nephrol.* (2021) 32:2147–52. doi: 10.1681/ASN.2021040480
22. Payne RP, Longet S, Austin JA, Skelly DT, Dejnirattisai W, Adele S, et al. Immunogenicity of standard and extended dosing intervals of BNT162b2 mRNA vaccine. *Cell.* (2021) 184:5699–714.e11. doi: 10.1016/j.cell.2021.10.011
23. Manisty C, Otter AD, Treibel TA, McKnight A, Altmann DM, Brooks T, et al. Antibody response to first BNT162b2 dose in previously SARS-CoV-2-infected individuals. *Lancet.* (2021) 397:1057–8. doi: 10.1016/S0140-6736(21)00501-8
24. Krammer F, Srivastava K, Alshammary H, Amoako AA, Awawda MH, Beach KF, et al. Antibody responses in seropositive persons after a single dose of SARS-CoV-2 mRNA vaccine. *N Engl J Med.* (2021) 384:1372–4. doi: 10.1056/NEJMc2101667
25. Saadat S, Rikhtegaran Tehrani Z, Logue J, Newman M, Frieman MB, Harris AD, et al. Binding and neutralization antibody titers after a single vaccine dose in health care workers previously infected with SARS-CoV-2. *JAMA.* (2021) 325:1467–9. doi: 10.1001/jama.2021.3341
26. Ebinger JE, Fert-Bober J, Printsev I, Wu M, Sun N, Prostko JC, et al. Antibody responses to the BNT162b2 mRNA vaccine in individuals previously infected with SARS-CoV-2. *Nat Med.* (2021) 27:981–4. doi: 10.1038/s41591-021-01325-6
27. Ortega N, Ribes M, Vidal M, Rubio R, Aguilar R, Williams S, et al. Seven-month kinetics of SARS-CoV-2 antibodies and role of pre-existing antibodies to human coronaviruses. *Nat Commun.* (2021) 12:4740. doi: 10.1038/s41467-021-24979-9
28. Goupil R, Benlarbi M, Beaubien-Souligny W, Nadeau-Fredette AC, Chatterjee D, Goyette G, et al. Short-term antibody response after 1 dose of BNT162b2 vaccine in patients receiving hemodialysis. *CMAJ.* (2021) 193:E793–800. doi: 10.1503/cmaj.210673
29. Richards NE, Keshavarz B, Workman LJ, Nelson MR, Platts-Mills TAE, Wilson JM. Comparison of SARS-CoV-2 antibody response by age among recipients of the BNT162b2 vs the mRNA-1273 vaccine. *JAMA Network Open.* (2021) 4:e2124331. doi: 10.1001/jamanetworkopen.2021.24331
30. Wang Z, Lorenzi JCC, Muecksch F, Finkin S, Viant C, Gaebler C, et al. Enhanced SARS-CoV-2 neutralization by dimeric IgA. *Sci Transl Med.* (2021) 13:577. doi: 10.1126/scitranslmed.abf1555
31. Mulley WR, Le ST, Ives KE. Primary seroresponses to double-dose compared with standard-dose hepatitis B vaccination in patients with chronic kidney disease: a systematic review and meta-analysis. *Nephrol Dial Transplant.* (2017) 32:136–43. doi: 10.1093/ndt/gfv443
32. Simon B, Rubey H, Treipl A, Gromann M, Hemedi B, Zehetmayer S, et al. Haemodialysis patients show a highly diminished antibody response after COVID-19 mRNA vaccination compared with healthy controls. *Nephrol Dial Transplant.* (2021) 36:1709–16. doi: 10.1093/ndt/gfab179
33. Levin EG, Lustig Y, Cohen C, Fluss R, Indenbaum V, Amit S, et al. Waning immune humoral response to BNT162b2 Covid-19 vaccine over 6 months. *N Engl J Med.* (2021) 385:e84. doi: 10.1056/NEJMoa2114583
34. Goldberg Y, Mandel M, Bar-On YM, Bodenheimer O, Freedman L, Haas EJ, et al. Waning immunity after the BNT162b2 vaccine in Israel. *N Engl J Med.* (2021) 385:e85. doi: 10.1056/NEJMoa2114228
35. Broseta JJ, Rodríguez-Espinosa D, Bedini JL, Rodríguez N, Maduell F. Antibody maintenance 3 months after complete messenger RNA COVID-19 vaccination in haemodialysis patients. *Nephrol Dial Transp.* (2021) 36:2340–1. doi: 10.1093/ndt/gfab272
36. Davidovic T, Schimpf J, Abbassi-Nik A, Stockinger R, Sprenger-Mähr H, Lhotta K, et al. Waning humoral response 6 months after SARS-CoV-2 vaccination with the mRNA-BNT162b2 vaccine in hemodialysis patients: time for a boost. *Kidney Int.* (2021) 100:1334–5. doi: 10.1016/j.kint.2021.10.006
37. Ducloux D, Colladant M, Chabannes M, Yannaraki M, Courivaud C. Humoral response after 3 doses of the BNT162b2 mRNA COVID-19 vaccine in patients on hemodialysis. *Kidney Int.* (2021) 100:702–4. doi: 10.1016/j.kint.2021.06.025
38. Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med.* (2021) 27:1205–11. doi: 10.1038/s41591-021-01377-8

39. Doria-Rose N, Suthar MS, Makowski M, O'Connell S, McDermott AB, Flach B, et al. Antibody persistence through 6 months after the second dose of mRNA-1273 vaccine for Covid-19. *N Engl J Med.* (2021) 384:2259–61. doi: 10.1056/NEJMc2103916
40. Alenquer M, Ferreira F, Lousa D, Valerio M, Medina-Lopes M, Bergman ML, et al. Signatures in SARS-CoV-2 spike protein conferring escape to neutralizing antibodies. *PLoS Pathog.* (2021) 17:e1009772. doi: 10.1371/journal.ppat.1009772
41. Gilbert PB, Montefiori DC, McDermott A, Fong Y, Benkeser D, Deng W, et al. Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy trial. *medRxiv*. (2021). doi: 10.1101/2021.08.09.21261290
42. Feng S, Phillips DJ, White T, Sayal H, Aley PK, Bibi S, et al. Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. *Nat Med.* (2021) 27:2032–40. doi: 10.1038/s41591-021-01540-1
43. Rincon-Arevalo H, Choi M, Stefanski A-L, Halleck F, Weber U, Szelinski F, et al. Impaired humoral immunity to SARS-CoV-2 BNT162b2 vaccine in kidney transplant recipients and dialysis patients. *Sci Immunol.* (2021) 6:eabj1031. doi: 10.1126/sciimmunol.abj1031
44. Sattler A, Schrezenmeier E, Weber UA, Potekhin A, Bachmann F, Straub-Hohenbleicher H, et al. Impaired humoral and cellular immunity after SARS-CoV-2 BNT162b2 (tozinameran) prime-boost vaccination in kidney transplant recipients. *J Clin Invest.* (2021) 131:e150175. doi: 10.1172/JCI150175

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Medicine

ORIGINAL RESEARCH
published: 15 February 2022
doi: 10.3389/fmed.2022.778464

Validation of the International IgA Nephropathy Prediction Tool in the Greek Registry of IgA Nephropathy

Marios Papasotiriou[1], Maria Stangou[2], Dimitris Chlorogiannis[3], Smaragdi Marinaki[4], Dimitrios Xydakis[5], Erasmia Sampani[2], Georgios Lioulios[2], Eleni Kapsia[4], Synodi Zerbala[6], Maria Koukoulaki[6], Georgios Moustakas[7], Stavros Fokas[7], Evangelia Dounousi[8], Anila Duni[8], Antonia Papadaki[9], Nikolaos Damianakis[9], Dimitra Bacharaki[10], Kostas Stylianou[11], Hariklia Gakiopoulou[12], George Liapis[12], Georgios Sakellaropoulos[3], Evangelos Papachristou[1], Ioannis Boletis[4], Aikaterini Papagianni[2] and Dimitrios S. Goumenos[1]*

[1] Department of Nephrology and Kidney Transplantation, University Hospital of Patras, Patras, Greece, [2] Department of Nephrology, Hippokration General Hospital, Aristotle University of Thassaloniki, Thessaloniki, Greece, [3] Department of Medical Physics and Bioinformatics, University of Patras, Patras, Greece, [4] Department of Nephrology and Kidney Transplantation, Laiko General Hospital of Athens, National and Kapodistrian University of Athens Medical School, Athens, Greece, [5] Department of Nephrology, General Hospital of Heraklion "Venizeleio-Pananeio", Heraklion, Greece, [6] Department of Nephrology, General Hospital of Nikaia, Piraeus, Greece, [7] Department of Nephrology, Gennimatas General Hospital of Athens, Athens, Greece, [8] Department of Nephrology, School of Medicine, University of Ioannina, Ioannina, Greece, [9] Department of Nephrology, General Hospital of Chania, Chania, Greece, [10] Second Department of Internal Medicine-Propaedeutic, Research Institute and Diabetes Centre, Attikon University Hospital, National and Kapodistrian University of Athens Medical School, Athens, Greece, [11] Department of Nephrology, University of Crete, Heraklion, Greece, [12] First Department of Pathology, National and Kapodistrian University of Athens Medical School, Athens, Greece

OPEN ACCESS

Edited by:
Yuji Nozaki,
Kindai University Hospital, Japan

Reviewed by:
Rajesh Nachiappa Ganesh,
Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), India
Vladimir Tesar,
Charles University, Czechia

***Correspondence:**
Marios Papasotiriou
mpapasotiriou@yahoo.com

Specialty section:
This article was submitted to Nephrology, a section of the journal Frontiers in Medicine

Received: *16 September 2021*
Accepted: *10 January 2022*
Published: *15 February 2022*

Citation:
Papasotiriou M, Stangou M, Chlorogiannis D, Marinaki S, Xydakis D, Sampani E, Lioulios G, Kapsia E, Zerbala S, Koukoulaki M, Moustakas G, Fokas S, Dounousi E, Duni A, Papadaki A, Damianakis N, Bacharaki D, Stylianou K, Gakiopoulou H, Liapis G, Sakellaropoulos G, Papachristou E, Boletis I, Papagianni A and Goumenos DS (2022) Validation of the International IgA Nephropathy Prediction Tool in the Greek Registry of IgA Nephropathy. Front. Med. 9:778464. doi: 10.3389/fmed.2022.778464

Background: Immunoglobulin A nephropathy (IgAN) is among the commonest glomerulonephritides in Greece and an important cause of end-stage kidney disease (ESKD) with an insidious chronic course. Thus, the recently published International IgAN prediction tool could potentially provide valuable risk stratification and guide the appropriate treatment module. This study aimed to externally validate this prediction tool using a patient cohort from the IgAN registry of the Greek Society of Nephrology.

Methods: We validated the predictive performance of the two full models (with or without race) derived from the International IgAN Prediction Tool study in the Greek Society of Nephrology registry of patients with IgAN using external validation of survival prediction models (Royston and Altman). The discrimination and calibration of the models were tested using the C-statistics and stratified analysis, coefficient of determination (R^2_D) for model fit, and the regression coefficient of the linear predictor (β_{PI}), respectively.

Results: The study included 264 patients with a median age of 39 (30–51) years where 65.2% are men. All patients were of Caucasian origin. The 5-year risk of the primary outcome (50% reduction in estimated glomerular filtration rate or ESKD) was 8%. The R^2_D for the full models with and without race when applied to our cohort was 39 and 35%, respectively, and both were higher than the reported R^2_D for the models applied to the original validation cohorts (26.3, 25.3, and 35.3%, respectively). Harrel's C statistic for the full model with race was 0.71, and for the model without race was 0.70. Renal survival curves in the subgroups (<16th, ∼16 to <50th, ∼50 to <84th, and >84th percentiles of

linear predictor) showed adequate separation. However, the calibration proved not to be acceptable for both the models, and the risk probability was overestimated by the model.

Conclusions: The two full models with or without race were shown to accurately distinguish the highest and higher risk patients from patients with low and intermediate risk for disease progression in the Greek registry of IgAN.

Keywords: IgAN prediction tool, IgAN disease progression, chronic kidney disease, immunosuppression, ACE inhibitors

INTRODUCTION

Immunoglobulin A nephropathy (IgAN) is considered to be the most frequent biopsy and proven type of glomerulonephritis with an estimated incidence of more than 1.5 per 100,000 persons every year. It can cause end-stage kidney disease (ESKD), in most instances, after a median disease course of more than 10 years (1). A particular feature of IgAN is the heterogeneous risk of progressive kidney function deterioration, with a 10-year risk of ESKD between 5 and 60%. Thus, IgAN treatment can be challenging, although Kidney Disease Improving Global Outcomes (KDIGO) guidelines recommend risk stratifying patients so that immunosuppressive treatment can be targeted to those at high-risk for disease progression; this stratification is based only on the degree of proteinuria which can be highly inaccurate. Until recently, there was no other tool available to accurately predict kidney disease progression (2). Nevertheless, a proportion of patients who presented with proteinuria of more than 1 g/day, and according to guidelines should be treated with immunosuppression therapy, have non-progressive disease. On the contrary, many patients with lower-grade proteinuria, who do not qualify for treatment, experience progressive disease (3, 4). This points out the necessity for an accurate clinical tool that predicts disease progression in IgAN.

Although there are well-accepted clinical and histological risk factors for kidney disease progression in IgAN, when these factors are used individually, they are unable, in many cases, to identify high-risk patients (3). Attempts in the past to establish a prediction model have not met widespread acceptance (5, 6). Although the Oxford MEST [mesangial (M) and endocapillary (E) hypercellularity, segmental sclerosis (S), and interstitial fibrosis/tubular atrophy (T)] histologic score in IgAN has been validated in international patient cohorts and is independently associated with a higher or lower risk of kidney function deterioration, it has only been recently incorporated into a risk prediction tool (7). This tool was developed by the International IgA Nephropathy Network, and it validated two versions: the full model without and with race. Although this tool has been validated and proved accurate in international cohorts of multi-ethnicity patients, there is still a paucity of evidence for its accuracy in single ethnic groups. Thus, this study aimed to validate the International IgA Nephropathy Prediction Tool using a large ethnic-based contemporary data set of patients with IgAN who were from Greece with fully available clinical, laboratory, and histological data.

METHODS

Patients

In this study, we included patients from the Greek Society of Nephrology IgAN registry (8). In this registry, patients with biopsy-proven IgAN are reported independently for research purposes from different nephrology departments across Greece. This cohort currently consists of 657 patients. Of these, only patients with available MEST scores and estimated glomerular filtration rate (eGFR) data with long-term follow-up after biopsy (over 1 year) were included in the final analysis. Furthermore, we included only those who were 18 years or older and who did not have established ESKD at the time of biopsy. This project was approved by the research ethics committee of the University Hospital of Patras, which waived patient's written informed consent for using their anonymized historical clinical data.

Definitions

Age, proteinuria, eGFR (using the Chronic Kidney Disease Epidemiology Collaboration formula), systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MAP = 1/3 x SBP + 2/3 x DBP), body mass index (BMI), prior use of medications that block the renin-angiotensin system blockers (RASBs, including angiotensin-converting enzyme inhibitors and angiotensin receptor blockers), and the use of immunosuppression were determined at the time of biopsy and during follow-up. The decline slope of eGFR was calculated using a linear regression line.

All patients included in this cohort were white Caucasians. Kidney biopsies were scored according to the Oxford MEST scoring system (**Supplementary Figure 1**) at the time of diagnosis by three pathologists who were not blinded to clinical data as a standard procedure (9). Crescent formation in kidney biopsies (C score) was not incorporated in the prediction tool, as according to the International IgAN tool proposed by Barbour et al. in the original derivation cohort, this variable did not correlate significantly with prognosis. The primary outcome was a composite of either ESKD (eGFR $<$ 15 mL/min/1.73 m^2, dialysis or kidney transplantation) or a reduction in eGFR below 50% of the value at biopsy for a period of more than 3 months, whichever occurred first. For validation, each covariate and outcome were defined exactly according to the original publication of Barbour et al. (10).

Prediction Models for External Validation

The prediction models for external validation were derived from the original publication of Barbour et al. (10) and described in detail by Zhang et al. (11).

Statistical Analysis

The initial step for the model validation was to calculate the linear predictor for each patient in our cohort based on the exact predictors and coefficient values. Then, we assessed the discrimination and calibration performance of the model according to Royston and Altman, and Zhang et al. (11, 12).

Discrimination was assessed first by estimating the regression coefficient on the linear predictor coefficient by fitting a Cox proportional hazards model for the full model without race and an interval format Cox proportional hazards model for the full model with race in our data set. If the slope on the linear predictor is >1, then the discrimination is better, and conservatively if it is <1, the discrimination is poorer. The model parameters for the calculation of the linear predictor were taken by the original publication (10) but the linear predictor itself was calculated using the equations as described in detail by Zhang et al. (11) for each patient of our data separately.

Second, Harrell's C-index of concordance or C-statistic was calculated to determine the ability of the model to discriminate between patients who have experienced the outcome of disease progression against those who did not. By definition, the C-statistic must lie between 0.5 and 1, with a general consensus that a C-statistic with an acceptable discrimination power is $\geq$ 0.65. In addition, the coefficient of determination (R^2_D) was also calculated using the method proposed by Royston and Sauerbrei (13).

Third, we divided the patients into risk groups, including $<$16th (low risk), approximately from 16 to $<$ 50th (intermediate risk), $\sim$50th to $<$ 84th (higher risk), and $>$ 84th (the highest risk) percentiles of the linear predictor from the full model without and with race. Subgroup analyses were performed, and survival curves were derived.

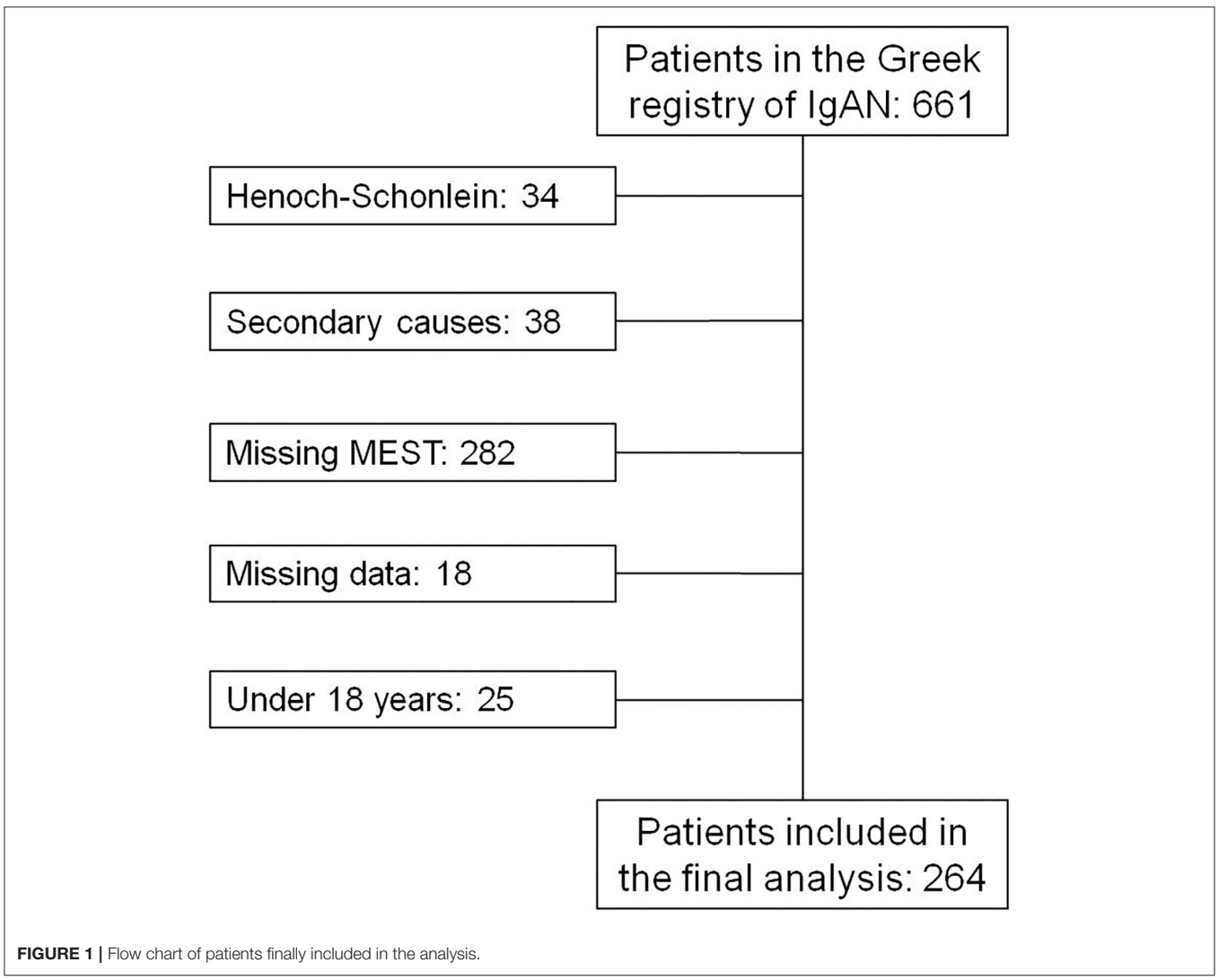


FIGURE 1 | Flow chart of patients finally included in the analysis.

As proposed by Royston et. al., the hazard ratios can be a sensible verification of model discrimination for comparing risk groups, in contrast to the *p*-values (12). Thus, hazard ratios were evaluated by fitting a Cox model with a dummy variable representing each risk group referring to the lowest risk group. When survival curves are more widely separated, the hazard ratio tends to be greater. For model calibration, the overall estimated regression coefficient of the linear predictor (β_{PI}) is the most precise estimate of relative global calibration and was calculated accordingly.

RESULTS

Clinical Characteristics and Outcome of Baseline Patients

The flow chart of the inclusion of the patients, in the final analysis, is presented in **Figure 1** and their clinical characteristics are presented in **Table 1**. There were 264 patients included in our analysis, all of Caucasian origin. In this cohort, the percentage of combined outcomes was 20.07% and among these, 12.9% reached ESKD and 13.6% showed a 50% decrease in eGFR. Mean follow-up was 8.5 (5–10.83) years.

The rate of RASB use was 44.5% at biopsy and 84.5% after biopsy while 46.2% received a form of immunosuppressive regimen during follow-up. Immunosuppression treatment prescription according to risk for disease progression is as follows; in the lower risk group (lower 16th percentile) the immunosuppression treatment prescription was 26.2%, in the intermediate-risk group (16–50th percentile), it was 33.3%, in the higher risk group (50–84th percentile), it was 58.9%, and in the highest risk group (upper 16th percentile), it was 66.7%. The immunosuppressive treatment options and protocols that were followed by each center varied depending on local practices and experience. These included 4 main treatment protocols i.e., oral steroid treatment based on a 6-month regimen of oral prednisone starting at 1 mg/kg/day for the first 2 months and with gradual tapering until the end of treatment at 6 months which was prescribed to 57 patients (6, 20, 21, and 10 patients in the lower, intermediate, high, and highest risk groups, respectively). Another option was the Pozzi regimen consisting of i.v. bolus injections of 1 g of methylprednisolone for 3 days each at months 1, 3, and 5, followed by an oral steroid of 0.5 mg/kg prednisone on alternate days for a total of 6 months which was followed by 24 patients (5, 5, 1, and 4 patients in the lower, intermediate, high, and highest risk groups, respectively); oral steroid treatment as mentioned in the first regimen plus azathioprine 100 mg/day for 6 months was followed by 21 patients (0, 1, 11, and 9 patients in the lower, intermediate, high and highest risk groups, respectively); and finally, i.v. bolus injections of 0.75 g/m^2 of body surface of cyclophosphamide every 4 weeks for 3 to 6 dosed in total plus 500 mg of i.v. methylprednisolone for 3 consecutive days plus oral prednisone of 1 mg/kg/day for 1 month with a maximum dose of 60 mg/day with gradual tapering over 4–6 months which was followed by 14 patients (0, 2, 8, and 4 patients in the lower, intermediate, high, and highest risk groups, respectively). Finally, another option that was scarcely used was the combination of oral prednisone with 1–2 g of mycophenolate mofetil which was followed by 6 patients (0, 2, 3, and 1 in the lower, intermediate, high, and highest risk groups, respectively). According to our data, 14 patients had IgA vasculitis and all were treated with IV cyclophosphamide. The use of immunosuppression ranged in older cohorts from 7.1 to 11.1% (11). Moreover, the distributions of other clinical parameters, including baseline kidney function, age, gender, and Oxford MEST histologic scores showed significant differences

TABLE 1 | Comparison of clinical and histological characteristics in the current and previously reported cohorts.

Characteristics	Reported derivation cohort	Reported validation cohort	This validation cohort
Number of patients	2,781	1,146	264
Follow up (yr)	4.8 (3.0–7.6)	5.8 (3.4–8.5)	8.5 (5–10.83)
Age at biopsy (yr)	35.6 (28.2–45.4)	34.8 (26.9–45.0)	39 (30–51)
Gender (M/F %)	1,608 (57.8) / 1,173 (42.2)	565 (49.3) / 581 (50.7)	172 (65.2) / 92 (34.8)
Race *n* (%)			
Caucasian	1,167 (42.0)	176 (15.5)	264 (100)
Chinese	1,021 (36.7)	292 (25.8)	-
Japanese	569 (20.5)	616 (54.4)	-
Other	22 (0.8)	49 (4.3)	-
sCr at biopsy (mmol/l)	1.04 (0.8–1.4)	84.0 (66.2–111.4) 0.95 (0.75–1.29)	1.2 (0.9–1.775)
eGFR at biopsy (ml/min/1.73 m^2)	83.0 (56.7–108.0)	89.7 (65.3–112.7)	61.09 (40.27–83.41)
<30, n (%)	142 (5.1)	37 (3.2)	43 (16.3)
30–60, *n* (%)	657 (23.6)	191 (16.7)	85 (32.2)
60–90, *n* (%)	800 (28.8)	350 (30.5)	83 (31.4)
>90, *n* (%)	1,182 (42.5)	568 (49.6)	53 (20.1)
MAP (mmHg)	96.7 (88.7–106.3)	93.3 (85.0–103.3)	100 (88.3–106.7)
Proteinuria			
<0.5, *n* (%)	383 (13.9)	221 (19.4)	42 (15.9)
0.5–1, *n* (%)	772 (28.1)	209 (18.3)	58 (21.9)
1–2, *n* (%)	817 (29.7)	352 (30.8)	84 (31.8)
2–3, *n* (%)	360 (13.1)	145 (12.7)	34 (12.9)
>3, *n* (%)	415 (15.1)	215 (18.8)	46 (17.4)
MEST score			
M1 (%)	1,054 (38.0)	481 (42.0)	186 (71)
E1 (%)	478 (17.3)	476 (41.5)	91 (34.7)
S1 (%)	2,137 (77)	912 (79.6)	154 (58.4)
T1 (%)	686 (24.7)	207 (18.1)	67 (25.6)
T2 (%)	128 (4.6)	122 (10.6)	11 (4.2)
RASB use, *n* (%)			
At biopsy	862 (32.4)	320 (30.0)	117 (44.5)
After biopsy	2,400 (86.7)	708 (66.4)	223 (84.5)
Immunosuppression			
After biopsy	1,209 (43.5)	359 (31.3)	122 (46.2)
Primary outcome			
50% eGFR decline	420 (15.1)	210 (18.3)	36 (13.6)
ESKD	372 (13.4)	155 (13.5)	34 (12.9)
Total primary outcome events	492 (17.7)	213 (18.6)	53 (20.07)

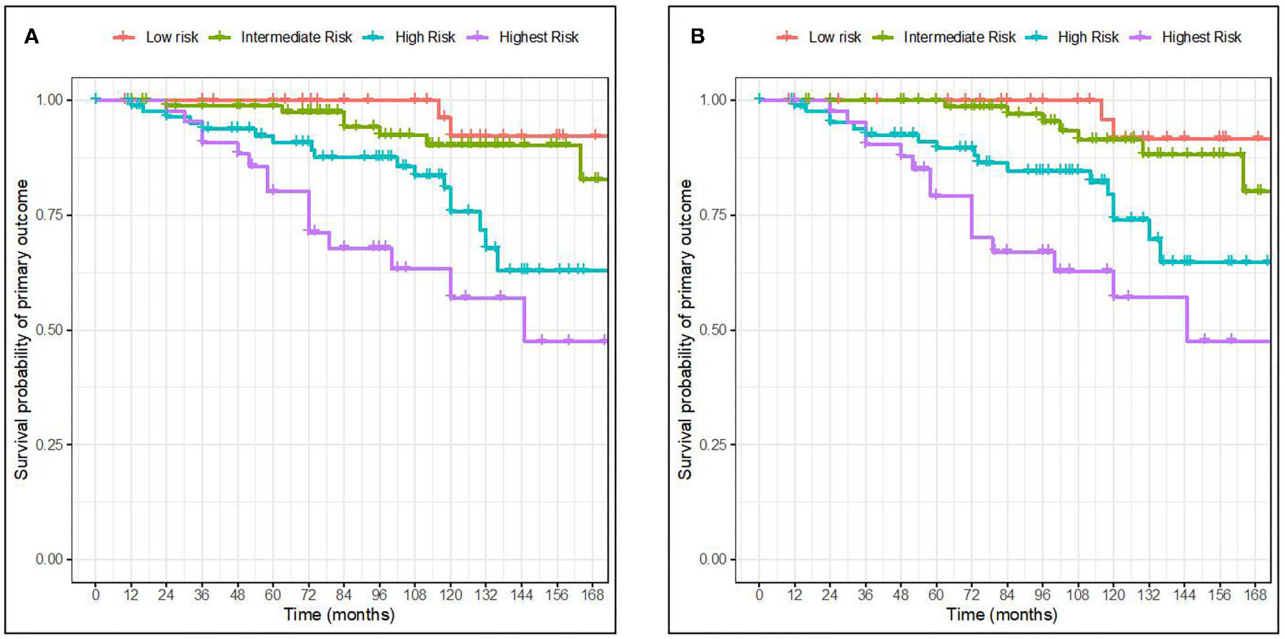


FIGURE 2 | Kaplan-Meier curves for survival probability of primary outcome in 4 risk groups based on the percentile of the linear predictor. Full model with race **(A)**. Full model without race **(B)**. The 4 risk groups were defined as <16th (low risk), ∼16th to <50th (intermediate risk), ∼50th to <84th (higher risk), and >84th (the highest risk) percentiles of the linear predictor from the full model without and with race, respectively.

between this and previously reported studies while proteinuria and blood pressure were similar.

Regression on Linear Predictor in Validation Data

The calibration slopes of linear prediction (β_{PI}) were 0.40 for the full model without race and 0.45 for the full model with race. Thus, discrimination appeared not to be preserved.

Measures of Discrimination and Model Fit

By applying the reported models to our current cohort, the C-statistic was calculated to be 0.70 for the model without race and 0.71 for the full model with race. In addition, R^2_D values were 35% for the full model without race and 39% for the full model with race indicating an increase compared to the ∼25% R^2_D values of the reported derivation cohorts. Thus, according to R^2_D, and contradictory to β_{PI}, a good performance of the model's fit was suggested.

Comparison of Risk Groups

Figures below show two Kaplan-Meier curves according to risk groups based on the percentiles of the linear predictor [<16th for low-risk group (red), ∼16th to <50th for intermediate-risk group (green), ∼50th to <84th for higher risk group (blue), and >84th for the highest risk group (purple)] (**Figure 2**).

The Kaplan-Meier curves of the risk groups were well separated for the high and highest risk groups of the two full models throughout the whole follow-up time. The low- and intermediate-risk groups however became more widely separated by 84 months of follow-up. When comparing our validation results with the ones from the original publication, the discrimination of groups was similar. Furthermore, the full model with race seemed more able to distinguish between the two lowest risk groups in our validation cohort.

The hazard ratios between risk groups were well-maintained. The predicted 5-year risks for patients in the 4 groups defined in our cohort were 27.5, 64.9, 98.4, and 99.9%, respectively for the full model without race, and 35, 73.7, 99.2, and 100%, respectively for the full model with race. Similarly, the eGFR decline slopes in the 4 groups were 1.67, 0.42, 1.18, and 1.77, respectively for the full model with race and 1.23, 0.80, 0.82, and 2.13, respectively for the full model without race. The clinical and histological characteristics of the patients across the risk groups based on the full model without and with race are presented in **Table 2**. Accordingly, we found that clinical characteristics were worse with increasing risk, defined as higher baseline proteinuria, worst kidney function, and more Oxford MEST lesions.

Model Calibration

Calibration generally describes the accuracy between the estimation or prediction of survival and the observed survival of the model as seen in the actual data. As previously mentioned by Royston et al. (12) a well-accepted approach to the validation of a model is to estimate the regression coefficient of the prognostic index (PI) in the validation dataset. Here, the PI was first computed for every individual in our cohort exactly as reported for the derivation cohort. Second, the estimate of the calibration slope or the regression coefficient for the PI

TABLE 2 | Clinical and histological characteristics of groups of patients according to risk stratification based on the full model without and with race.

Full model without race	**Group 1 (lower 16th percentile) *n* = 42**	**Group 2 (16–50th percentile) *n* = 90**	**Group 3 (50–84th percentile) *n* = 90**	**Group 4 (upper 16th percentile) *n* = 42**	***P*-value**
Biopsy age	30 (21.75–42)	39 (30–52.25)	42 (33–52.25)	40.5 (36.5–57.25)	<0.001
Systolic BP	105 (100–116.5)	130 (120–140)	145 (130–150)	157.5 (141.5–167.3)	<0.001
Diastolic BP	69 (60–75)	80 (75–85)	85 (80–90)	90 (80–98)	<0.001
eGFR diagnosis	99.08 (74.99–120.4)	69.35 (52.1–91.9)	50.5 (33.7–67.9)	31 (23.3–46.3)	<0.001
sCr diagnosis	0.85 (0.8–0.9)	1.06 (0.9–1.36)	1.4 (1.1–2.03)	1.95 (1.4–2.63)	<0.001
Proteinuria diagnosis	620 (292.5–1,063)	900 (490–1,500)	1,893 (1,100–3,250)	2,329 (1,788–3,100)	<0.001
M (0/1)	20 (47.6%)/22 (52.4%)	25 (27.8%)/65 (72.2%)	23 (25.6%)/65 (72.2%)	8 (19.1%)/34 (80.9%)	0.023
E (0/1)	36 (85.7%)/6 (42.3%)	63 (70%)/27 (30%)	51 (56.7%)/37 (41.1%)	21 (50%)/21 (50%)	0.002
S (0/1)	29 (69%)/13 (31%)	41 (45.6%)/49 (54.4%)	28 (31.1%)/60 (66.7%)	11 (26.2%)/31 (73.8%)	<0.001
T (0/1/2)	41 (97.6%)/1 (2.4%)/0(0%)	85 (94.4%)/1 (1.1%)/4 (4.4%)	52 (57.8%)/29 (32.2%)/7 (7.8%)	6 (14.3%)/36 (85.7%)/0(0%)	<0.001
Full model with race	*n* = 42	*n* = 90	*n* = 90	*n* = 42	
Biopsy age	31.5 (22–42.5)	39 (30–53)	41 (32–52)	45.5 (38–58.25)	0.001
Systolic BP	105 (100–118.5)	128 (120–140)	140 (130–150.5)	154.5 (146.8–167)	<0.001
Diastolic BP	69.5 (60–75)	80 (70–84.5)	85 (80–90)	90 (80.75–98)	<0.001
eGFR diagnosis	96.6 (76.3–114.1)	71 (57.3–92.2)	48.9 (33.7–65.7)	29.3 (21.4–42.9)	<0.001
sCr diagnosis	0.9 (0.8–0.9)	1.02 (0.9–1.2)	1.5 (1.2–1.95)	2.05 (1.65–2.7)	<0.001
Proteinuria diagnosis	500 (252–825)	900 (500–1500)	1800 (1200–2850)	2566 (1800–3937)	<0.001
M (0/1)	17 (40.5%)/25 (59.5%)	28 (31.1%)/61 (67.8%)	25 (27.8%)/64 (71.1%)	3 (7.1%)/36 (85.7%)	0.06
E (0/1)	33 (78.6%)/9 (21.4%)	64 (71.1%)/25 (27.8%)	54 (60%)/35 (38.9%)	20 (47.6%)/22 (52.4%)	0.009
S (0/1)	27 (64.3%)/15 (35.7%)	45 (50%)/44 (48.9%)	28 (31.1%)/61 (67.8%)	9 (21.4%)/33 (78.6%)	<0.001
T (0/1/2)	41 (97.6%)/1 (2.4%)/0 (0%)	84 (93.3%)/1 (1.1%)/4 (4.4%)	53 (58.9%)/29 (32.2%)/7 (7.8%)	6 (14.3%)/36 (85.7%)/0 (0%)	<0.001

The test used for comparison of continuous variables was ANOVA, and for categorical variables, the Chi-square test was used.

was calculated for the validation dataset. The overall estimate of the β of the PI is the most precise estimate of the relative global calibration. In our analysis, the estimate of the β_{PI} for the full model with and without race was calculated to be 0.40 (SE = 0.08) and 0.45 (SE = 0.08), respectively, which are far from 1. Thus, it appears that both the models failed to show adequate calibration in this validation cohort and thus cannot accurately predict the 5-year risk for ESKD. This is also apparent in the difference between the predicted mean 5-year risk as calculated from the model (**Table 3**) and the observed survival as shown in the Kaplan-Meier curves (**Figure 2**), in which the model overestimates the mean 5-year risk between all risk groups.

DISCUSSION

In this study, we validated two risk-prediction models which accurately predict a 50% decline in eGFR or ESKD in patients with IgAN using the available data set from the national IgAN registry of the Greek society of Nephrology (8). From this data set, we extracted and used the clinical and histological information from patients with available MEST scores. In this study, we examined the value and precision in reproducing the predicted risk of a 50% decline in eGFR or ESKD using the already available IgAN international prediction tool from an ethnically homogeneous cohort. Moreover, we examined the validity for both prediction models; the one that

TABLE 3 | Discrimination measures in the current and reported cohorts.

Measure	Hazard ratio	Mean predicted 5y risk, %	eGFR decline slope
Full model without race			
Low risk group	Reference	27.5	−1.67
Intermediate risk group	2.15 (0.6–7.6)	64.9	−0.42
High risk group	4.24 (1.2–14.29)	98.4	−1.18
Highest risk group	9.05 (2.6–30)	99.9	−1.77
Full model with race			
Low risk group	Reference	35.0	−1.23
Intermediate risk group	1.82 (0.5–6.53)	73.7	−0.80
High risk group	4.55 (1.35–15.26)	99.2	−0.82
Highest risk group	8.66 (2.54–29.5)	100	−2.13

includes race/ethnicity in calculating risk and the other without race/ethnicity. Both the models could not accurately capture and predict the 5-year risk; however, they were able to accurately distinguish the highest and higher risk patients from patients with low and intermediate risk.

As the diagnosis of IgAN is only established after a successful renal biopsy containing more than 10 glomeruli, a prediction model with standard histological characteristics would help increase the accuracy of the model. Moreover, there are studies based on urine and serum biomarkers that reflect kidney fibrosis and ongoing disease progression. Nevertheless, the use of such markers has not proven their efficacy in everyday clinical practice (14). Furthermore, the established and histologically reproducible MEST score has a proven value in the long-term prediction of disease progression (15). On the other hand, although some other models for predicting disease progression were developed containing histological variables, these models were either based on a relatively small or in a single patient population (6, 16, 17). In this context, recently two full models combining clinical and histological variables (Oxford MEST score) were derived and validated in two multi-ethnic cohorts (10). These models contain well-established factors for IgAN progression which can easily and consistently be obtained.

Our results point out that both prediction models are fairly suitable for implementation in ethnic Greeks and improve kidney function risk stratification and subsequent decision-making for appropriate clinical treatment. Our analysis showed that survival curves of different risk groups were adequately separated in both the models. Nevertheless, the eGFR decline slope was not consistently larger among the lower, intermediate, and higher risk groups, as it was for the highest risk group. However, both the models showed that the prediction risk over 5 years was extremely overestimated in our patients. Overall, we suggest using the full model without race for further clinical utility assessment and decision-making for pharmacological interventions in the Greek population with IgAN.

The use of immunosuppressive treatment after biopsy in any risk group of our cohort reached 46.2% which is higher than that used in the originally reported validation cohort (10) as well as in another Asian-Caucasian cohort which was used for external validation of the risk-prediction model (11). Moreover, 26.2% in the lowest and 33.3% of patients in the intermediated risk group received immunosuppressive treatment as well. This is in accordance with other studies which point that a large proportion of up to 75% of patients are over-treated with immunosuppression despite having a non-progressive disease even when its value has not been proved in large prospective randomized trials (18–21). On the contrary, the majority, but not all of the patients that showed higher or the highest risk of disease progression received immunosuppressive treatment consisting either of corticosteroids alone (per o.s. or i.v.) or with a combination with either azathioprine or cyclophosphamide. Although the exact risk stratification threshold for immunosuppression initiation is yet to be determined based on the risk-benefit ratio, having a reliable, easy-to-use tool, will facilitate clinical trial design by focusing on different treatment regimens according to the individualized patient risk of disease progression. Accordingly, this will eventually offer and configurate the appropriate risk-based treatment protocols.

Although the IgAN risk tool was evaluated in an international, multiethnic cohort addressing issues of previous studies, such as small size cohorts with only a few patients across the spectrum of disease activity and inter-ethnicity difference, we believe that our cohort further enhances the validity of previous results. That is because our cohort consists of an adequate number of patients that covers the whole spectrum of disease activity and with a long follow-up of more than 8 years. Furthermore, as the original prediction risk tool accommodates differences across different ethnic groups, we believe that the use of our group of patients highlights not only specific similarities but also disparities compared to the international population (7).

Our patients showed a similar burden of total primary outcome events in comparison to both the originally reported validation cohort (10) as well as in another Asian-Caucasian cohort (11). Nevertheless, the total follow-up of these events that were captured was significantly higher in our cohort. This was despite a significantly lower median eGFR at diagnosis and a higher proportion of patients with established chronic kidney disease stage III or worst. Furthermore, this was not accompanied by significant differences in conservative renin-angiotensin-aldosterone system inhibitor (RAASi) treatment trends after diagnosis, in comparison to the other validation cohorts (10, 11). In comparison to the original validation cohort (10), RAASi initiation before diagnosis was higher but in comparison to the cohort by Zhang et al. (11), it was significantly lower. This was probably due to different treatment approaches in patients of the current and older eras that were included in our cohort. In any case, this strengthens the analysis as our cohort represents both current and older treatment regimens. Concerning immunosuppression, a slightly higher percentage of our patients received such treatment in comparison to both previously mentioned validation cohorts. Thus, the

unexpected better 5-year survival of our cohort could in part be attributed not only to different treatment decisions and trends but also highlights the differences in disease progression among ethnic groups (22).

The strength of our study is that it used a population of patients with long-term follow-up, far more than the original and other validation cohorts (7, 23). This gives us the advantage of capturing those patients with silent and gradual but ominous disease progression well beyond after diagnosis which is an IgAN characteristic (24). However, a limitation of our study is the exclusion from the final cohort of those patients who did not have an available Oxford MEST histologic score. This group of patients is currently the largest in the IgAN registry of the Greek society of Nephrology which means that we have missed some intermediate-risk patients who showed a gradual disease progression. Moreover, Group 2 and Group 3 have 4 and 7 patients, each with T2 lesions in biopsy thus highlighting significant interstitial fibrosis and tubular atrophy while Group 4 does not have a patient with T2 lesion (please refer to **Table 2**), nevertheless, the percentage of patients in total with T2 lesions in our cohort is identical to that of the original derivation cohort (4.6 vs. 4.2% in our cohort); thus, we consider that this finding cannot compromise our results. Another limitation of the prediction model is that it can be used only for a relatively short-term prognosis (up to 8 years), considering that IgAN has a long-term evolution.

In conclusion, we validated the full prediction models for risk stratification of patients with IgAN. These models showed an inferior performance on a personalized risk assessment in comparison to one of the original derivation cohorts. Specifically, this tool can precisely stratify Greek patients with IgAN into four major risk groups (low, intermediate, high, and highest risk) but without accurately predicting their 5-year kidney function. Overall, this tool may help discriminate high-risk patients who will benefit from immunosuppression treatment and avoid such interventions in those with low risk for disease progression. However, it is important to re-validate this tool in a larger population to further investigate its accuracy which emphasizes the need to expand the Greek national and other international IgAN registries.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University Hospital of Patras. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DG and MP: conceptualization. GS and MP: methodology. DC: software. DC and GS: validation. DC, GS, and MP: formal analysis. HG and GL: investigation. SM, DX, ES, GL, EK, SZ, MK, GM, SF, ED, AD, APd, ND, DB, and KS: resources and data curation. MP: writing—original draft preparation. MP, MS, and DC: writing—review and editing. MP and EP: visualization. EP, IB, and APg: supervision. DG: project administration. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by a grant from the Hellenic Society of Nephrology.

ACKNOWLEDGMENTS

We thank all the participating centers and their patients for their cooperation.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.778464/full#supplementary-material

Supplementary Figure 1 | Representative mesangial [M] and endocapillary [E] hypercellularity, segmental sclerosis [S], and interstitial fibrosis/tubular atrophy [T] (MEST) score kidney biopsy pictures. **(A)** MEST: M1; Glomerulus with global severe mesangial hypercellularity, HE x 400. **(B)** MEST: E1; Mesangial hypercellularity and segmental endocapillary hypercellularity, PAS x 400. **(C)** MEST: S1; Segmental glomerulosclerosis with adhesion to Bowman's capsule that lies closer to the vascular pole (arrow), HE x 400. **(D)** MEST: S1; Segmental glomerulosclerosis with adhesion to Bowman's capsule that lies close to the tubular pole (arrow), Masson x 400. **(E)** MEST: T0; Minimal Interstitial fibrosis in a patient with IgA nephropathy, Masson x 200. **(F)** MEST: T1; Focus of tubular atrophy and interstitial fibrosis in a patient with IgA nephropathy whose biopsy showed moderate but no more than 50% tubular atrophy or interstitial fibrosis (arrow), PAS x 100. **(G)** MEST: T2; Interstitial fibrosis and tubular atrophy in a patient with IgA nephropathy whose biopsy showed diffuse interstitial fibrosis, Masson x 200. **(H)** MEST: C1; Endocapillary hypercellularity with crescent formation (arrow), HE x 400. HE, hematoxylin and eosin staining; PAS, periodic acid-Schiff staining.

REFERENCES

1. Lai KN, Tang SC, Schena FP, Novak J, Tomino Y, Fogo AB, et al. Iga nephropathy. *Nat Rev Dis Primers.* (2016) 2:16001. doi: 10.1038/nrdp.2016.1
2. Radhakrishnan J, Cattran DC. The kdigo practice guideline on glomerulonephritis: Reading between the (guide)lines–application to the individual patient. *Kidney Int.* (2012) 82:840–56. doi: 10.1038/ki.2012.280
3. Barbour SJ, Reich HN. Risk stratification of patients with iga nephropathy. *Am J Kidney Dis.* (2012) 59:865–73. doi: 10.1053/j.ajkd.2012.02.326
4. Bartosik LP, Lajoie G, Sugar L, Cattran DC. Predicting progression in iga nephropathy. *Am J Kidney Dis.* (2001) 38:728–35. doi: 10.1053/ajkd.2001.27689
5. Wakai K, Kawamura T, Endoh M, Kojima M, Tomino Y, Tamakoshi A, et al. A scoring system to predict renal outcome in iga nephropathy: from

a nationwide prospective study. *Nephrol Dial Transplant.* (2006) 21:2800–8. doi: 10.1093/ndt/gfl342
6. Goto M, Kawamura T, Wakai K, Ando M, Endoh M, Tomino Y. Risk stratification for progression of iga nephropathy using a decision tree induction algorithm. *Nephrol Dial Transplant.* (2009) 24:1242–7. doi: 10.1093/ndt/gfn610
7. Barbour SJ, Canney M, Coppo R, Zhang H, Liu ZH, Suzuki Y, et al. Improving treatment decisions using personalized risk assessment from the international iga nephropathy prediction tool. *Kidney Int.* (2020) 98:1009–19. doi: 10.1016/j.kint.2020.04.042
8. Stangou M, Papasotiriou M, Xydakis D, Oikonomaki T, Marinaki S, Zerbala S, et al. Iga nephropathy in greece: data from the registry of the hellenic society of nephrology. *Clin Kidney J.* (2018) 11:38–45. doi: 10.1093/ckj/sfx076
9. Working Group of the International Ig ANN, the Renal Pathology S, Cattran DC, Coppo R, Cook HT, Feehally J, et al. The oxford classification of iga nephropathy: Rationale, clinicopathological correlations, and classification. *Kidney Int.* (2009) 76:534–45. doi: 10.1038/ki.2009.243
10. Barbour SJ, Coppo R, Zhang H, Liu ZH, Suzuki Y, Matsuzaki K, et al. Evaluating a new international risk-prediction tool in iga nephropathy. *JAMA Intern Med.* (2019) 179:942–52. doi: 10.1001/jamainternmed.2019.0600
11. Zhang Y, Guo L, Wang Z, Wang J, Er L, Barbour SJ, et al. External validation of international risk-prediction models of iga nephropathy in an asian-caucasian cohort. *Kidney Int Rep.* (2020) 5:1753–63. doi: 10.1016/j.ekir.2020.07.036
12. Royston P, Altman DG. External validation of a cox prognostic model: principles and methods. *BMC Med Res Methodol.* (2013) 13:33. doi: 10.1186/1471-2288-13-33
13. Royston P, Sauerbrei W. A new measure of prognostic separation in survival data. *Stat Med.* (2004) 23:723–48. doi: 10.1002/sim.1621
14. Genovese F, Boor P, Papasotiriou M, Leeming DJ, Karsdal MA, Floege J. Turnover of type iii collagen reflects disease severity and is associated with progression and microinflammation in patients with iga nephropathy. *Nephrol Dial Transplant.* (2016) 31:472–9. doi: 10.1093/ndt/gfv301
15. Coppo R, D'Arrigo G, Tripepi G, Russo ML, Roberts ISD, Bellur S, et al. Is there long-term value of pathology scoring in immunoglobulin a nephropathy? a validation study of the oxford classification for iga nephropathy (valiga) update. *Nephrol Dial Transplant.* (2020) 35:1002–9. doi: 10.1093/ndt/gfy302
16. Goto M, Wakai K, Kawamura T, Ando M, Endoh M, Tomino Y, et al. scoring system to predict renal outcome in iga nephropathy: a nationwide 10-year prospective cohort study. *Nephrol Dial Transplant.* (2009) 24:3068–74. doi: 10.1093/ndt/gfp273
17. Kalliakmani P, Komninakis D, Gerolymos M, Papasotiriou M, Savvidaki E, Goumenos DS. Treatment of iga nephropathy based on the severity of clinical and histological features. *Saudi J Kidney Dis Transpl.* (2015) 26:516–25. doi: 10.4103/1319-2442.157356
18. Pozzi C, Bolasco PG, Fogazzi GB, Andrulli S, Altieri P, Ponticelli C, et al. Corticosteroids in iga nephropathy: a randomised controlled trial. *Lancet.* (1999) 353:883–7. doi: 10.1016/S0140-6736(98)03563-6
19. Manno C, Torres DD, Rossini M, Pesce F, Schena FP. Randomized controlled clinical trial of corticosteroids plus ace-inhibitors with long-term follow-up in proteinuric iga nephropathy. *Nephrol Dial Transplant.* (2009) 24:3694–701. doi: 10.1093/ndt/gfp356
20. Lv J, Zhang H, Wong MG, Jardine MJ, Hladunewich M, Jha V, et al. Effect of oral methylprednisolone on clinical outcomes in patients with iga nephropathy: the testing randomized clinical trial. *JAMA.* (2017) 318:432–42. doi: 10.1001/jama.2017.9362
21. Rauen T, Eitner F, Fitzner C, Sommerer C, Zeier M, Otte B, et al. Intensive supportive care plus immunosuppression in iga nephropathy. *N Engl J Med.* (2015) 373:2225–36. doi: 10.1056/NEJMoa1415463
22. Yeo SC, Goh SM, Barratt J. Is immunoglobulin a nephropathy different in different ethnic populations? *Nephrology.* (2019) 24:885–95. doi: 10.1111/nep.13592
23. Barbour SJ, Coppo R, Er L, Russo ML, Liu ZH, Ding J, et al. Updating the international iga nephropathy prediction tool for use in children. *Kidney Int.* (2020) 99:1439-50. doi: 10.1016/j.kint.2020.10.033
24. Floege J. A new tool to predict the risk of progression in iga nephropathy. *Kidney Int.* (2019) 96:808–9. doi: 10.1016/j.kint.2019.07.009

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership