Supplementary Methods

Cytomegalovirus Infection Determines Microvascular Inflammation Risk beyond Alloimmunity in Kidney Transplantation

Thomas Vanhoutte, Matthias Diebold, Karolien Wellekens, Priyanka Koshi, Jasper Callemeyn, Bertrand Chauveau, Tim Debyser, Steffi De Pelsmaeker, Marie-Paule Emonds, Petra Hruba, Dirk Kuypers, Christophe Masset, Angelica Pagliazzi, Elisabeth Puchhammer-Stöckl, Arthur Vranken, Stefan Schaub, Aleksandar Senev, Elisabet Van Loon, Thibaut Vaulet, Hannes Vietzen, Maarten Coemans, Ondrej Viklicky, Georg A. Böhmig, Maarten Naesens

Table 1. Detailed description of the cohorts

Cohort	*Data collection and ethics*	*Biopsy mechanism*	*C4d assessment*	*HLA-DSA detection*
Basel	The Basel cohort included 507 consecutive adult kidney transplant recipients transplanted at the University Hospital Basel between 2015 and 2021. Clinical and laboratory data were prospectively collected. All participants provided written informed consent, and the study was approved by the local ethics committee (EKNZ Project ID 2022-01789).	Indication biopsies were performed for graft dysfunction, proteinuria, detection of donor-specific antibodies, and/or elevated urinary chemokine (CXCL10) levels.	C4d staining was performed by IHC on paraffin-embedded tissue sections. Peritubular capillary staining was graded 0–3 according to Banff; scores ≥1 were considered positive.	HLA-antibodies were assessed pre- and post-transplant by Luminex single antigen assays with a MFI cutoff of >500 across all loci (A, B, C, DRB1, DRB3/4/5, DQ, DP). Screening was performed at 1 year post-transplantation, every 2 years thereafter (i.e., years 3, 5, 7, etc.), and whenever a biopsy demonstrated features of MVI.
Leuven	The Leuven cohort comprised 1,891 consecutive adult kidney transplants followed at University Hospitals Leuven between 2004 and 2021. Clinical and laboratory data were prospectively collected. Most participants provided written informed consent; for a subset, the ethics committee approved a waiver under Belgian law (May 7, 2004) for studies using routinely collected clinical data. The study was approved by the Ethics Committee of University Hospitals Leuven (S64006).	Biopsies were performed when clinically indicated or at specific time protocol biopsy time points (3, 12, and 24 months post transplantation). Recipients transplanted before October 2005, November 2008 and January 2010 underwent protocol biopsies at 48, 36, and 60 months, respectively.	C4d staining of peritubular capillaries was assessed by IHC on frozen tissue and graded from 0–3. A threshold of ≥2 defined C4d positivity, which showed 89% concordance with IF.	HLA-DSA were screened pre- and post-transplant and at each biopsy using a Luminex-based single antigen bead assay (Immucor®) for HLA-A, -B, -C, -DR, -DQ, and -DP. After Luminex replaced ELISA in 2008, prospectively biobanked sera were re-analyzed. Positivity was defined at MFI >500.
Prague	The Prague cohort included 1,005 adult kidney transplants followed at the Institute for Clinical and Experimental Medicine (IKEM), between 2019 and 2023. The study was approved by the institutional review of the Institute for Clinical and Experimental Medicine in Prague (A 13-02-01 (83/13)).	Biopsies were performed on indication, or per protocol, at 3 months post-transplant.	C4d was assessed by IF on fresh-frozen tissue. Three- to four-micrometer cryostat sections were incubated with anti-C4d and evaluated for peritubular capillary staining. The extent of staining was graded according to the Banff scoring	Donor-specific HLA-antibodies were screened using a Luminex single antigen bead (SAB) assay (One Lambda) with a positivity cutoff of MFI >2000.

			system (0–3; 0, none; 1, 1–10%; 2, 10–50%; 3, >50%), with scores ≥2 classified as C4d-positive.	
Vienna	The Vienna cohort comprised 1,097 adult kidney transplants primarily followed at the Medical University of Vienna between 2013 and 2023. Data collection was approved by the Institutional Review Board of the Medical University of Vienna (EK-Nr. 55 267/2011).	Biopsies were performed for indication or per protocol, as part of the routine follow-up at 3 months, 12 months, and/or 3 years post-transplant.	C4d staining was performed by IHC on paraffin-embedded tissue sections. Peritubular capillary staining was graded 0–3 according to Banff; scores ≥1 were considered positive.	HLA-antibodies were tested by Luminex single antigen assays with a MFI cutoff of >1000. Circulating de novo HLA donor-specific antibodies (DSA) were assessed across all loci (A, B, C, DRB1, DRB3/4/5, DQ, DP). Screening was performed at 3 months, at 1 year post-transplantation, and every 2 years thereafter (i.e., years 3, 5, etc.), and in some cases at the time of protocol or index biopsies.

Abbreviations: C4d, complement split product C4d; CXCL10, C–X–C motif chemokine ligand 10; DSA, donor-specific antibody; ELISA, enzyme-linked immunosorbent assay; HLA, human leukocyte antigen; IF, immunofluorescence; IHC, immunohistochemistry; IKEM, Institute for Clinical and Experimental Medicine; MFI, mean fluorescence intensity; SAB, single antigen bead.

Table 2: STROBE checklist

STROBE Statement: Checklist of items that should be included in reports of cohort studies

	Item No	*Recommendation*	*Page No*
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Abstract, 5
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Abstract, 5
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6–7
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	7–8, 20, statistical analysis (22–24),
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7–8, 20, statistical analysis (22–24), Supplementary methods
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	7–8, 20, statistical analysis (22–24), Supplementary methods
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	Not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Methods (20–23), statistical analysis (22–24)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Methods (20–23), Supplementary methods

Bias		9	Describe any efforts to address potential sources of bias	Statistical analysis (22–24)
Study size		10	Explain how the study size was arrived at	Methods (20–23), Figure 1
Quantitative variables		11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Statistical analysis (22–24)
Statistical methods		12	(*a*) Describe all statistical methods, including those used to control for confounding	Statistical analysis (22–24)
			(*b*) Describe any methods used to examine subgroups and interactions	Statistical analysis (22–24)
			(*c*) Explain how missing data were addressed	Statistical analysis (22–24)
			(*d*) If applicable, explain how loss to follow-up was addressed	Statistical analysis (22–24)
			(*e*) Describe any sensitivity analyses	Statistical analysis (22–24)
Results				
Participants		13*	(a) Report numbers of individuals at each stage of study—e.g. numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analyzed	8–13, Figure 1
			(b) Give reasons for non-participation at each stage	8–13, Figure 1
			(c) Consider use of a flow diagram	Figure 1
Descriptive data		14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8–13, Table 1
			(b) Indicate number of participants with missing data for each variable of interest	Table 1
			(c) Summarize follow-up time (e.g., average and total amount)	8
Outcome data		15*	Report numbers of outcome events or summary measures over time	8–13
Main results	16		(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8–13, Tables 2–5
			(*b*) Report category boundaries when continuous variables were categorized	8–13, Table 1

		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable
Other analyses	17	Report other analyses done—e.g. analyses of subgroups and interactions, and sensitivity analyses	8–13, statistical analysis (22–24)
Discussion			
Key results	18	Summarize key results with Reference to study objectives	14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	18
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	14–19
Generalizability	21	Discuss the generalizability (external validity) of the study results	14–19
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Funding (43)

*Give information separately for exposed and unexposed groups.