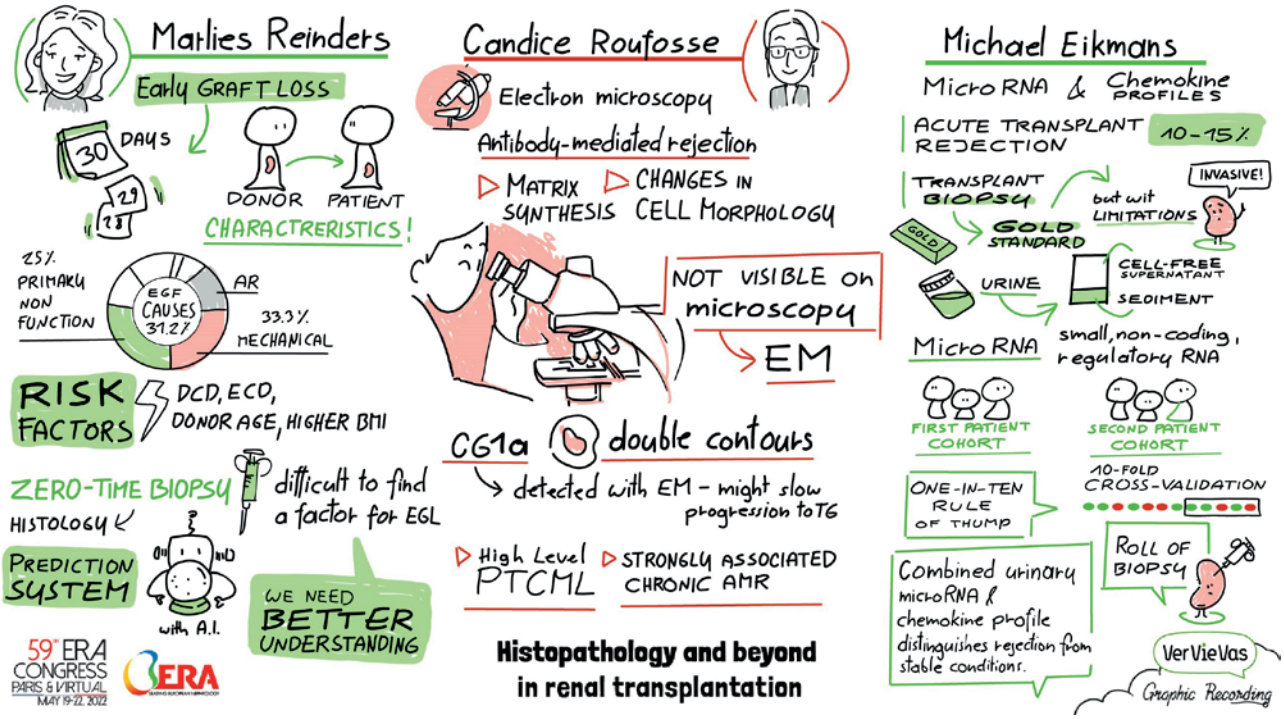
Symposium 6.4
Histopathology and beyond in Renal Transplantation



Determination of early graft loss: what we learn from histopathological findings?

Marlies Reinders, Netherlands

Early graft loss (EGL) is defined as graft nephrectomy or permanent loss of kidney transplant function resulting in dialysis dependence or death up to one year after transplantation. A notable inconsistency in the definition of the period for EGL, ranging from 30 days to 1 year following transplantation hinders interstudy reproducibility.

The factors associated with EGL include organ quality, surgical factors, delayed graft function (DGF), and acute rejection, while late outcomes encompass graft and recipient factors, chronic rejection, diabetes, cardiovascular disease, recurrence of primary kidney disease, and adherence to immunosuppressive therapy. Recipients with EGL have 12 times increased risk of death within the first year. Current trends in the renal transplant population show a rising incidence of a transplant from deceased donors with circulatory death, higher body mass index and prevalence of diabetes among recipients, prolonged cold ischemia time, higher rate of panel reactive

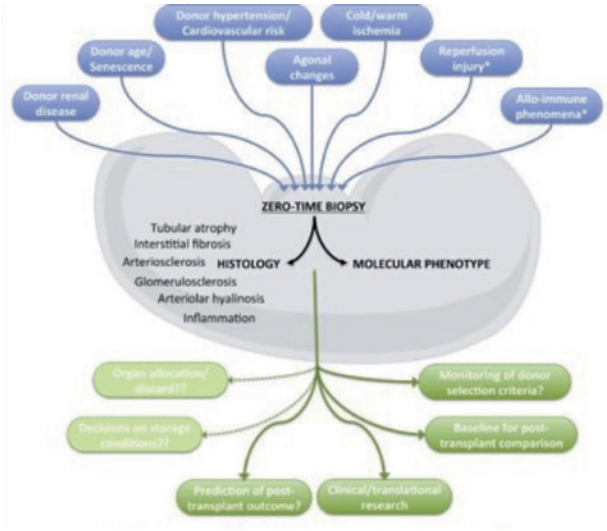


Figure 1.
Applications of performing zero-time biopsies and factors affecting histology and molecular phenotype (from ref. 6)

antibody, HLA mismatch, and DGF.

European data show a considerable improvement in terms of EGL from 1986 till 2000, but we witness a stagnation in the last decades thus calling for innovation in this field. Over half of the graft loss cases are due to recipient death, mainly caused by malignancy, infections, and cardiovascular disease. The major causes of graft failure include non-specific chronic injury, acute and chronic rejection, and mechanical injury. Graft loss within the first year of transplantation is mainly caused by mechanical injury, primary non-function, and acute rejection, while later failures are mostly related to non-specific chronic injury. A compelling number of lost transplants show overlapping histopathological features of multiple active and chronic lesions and their prevalence increases over time. Late loss is characterized by a high prevalence of arteriolar hyalinosis, interstitial fibrosis, and tubular damage.

The elderly recipients exhibit a 90% one-year survival, with infections and cardiovascular events being the major causes of death. The independent risk factors for death or graft failure in this population are arrhythmia, left-ventricular ejection fraction under 56%, HLA antibodies, a deceased donor from cardiovascular cause, and acute rejection. These results underline the importance of cardiac evaluation and immunosuppression optimization in this population.

Graft biopsies performed at the time of transplantation ("zero biopsies") provide valuable insight into risk factors for posttransplant events and serve as a baseline for comparison with posttransplant histology. However, the predictive performance of individual histological lesions and composite scores for the posttransplant outcome is at best moderate and no single histological lesion or composite score is sufficiently robust to be included in algorithms for kidney discard. Novel techniques, including single-cell genomics, should also be considered as a tool to detect potential therapeutic targets to prevent rejections in the future.

The importance of electron microscopy in diagnosing renal transplant rejection

Candice Roufosse, United Kingdom

Electron microscopy (EM) is extensively used in native kidney histopathological analysis which significantly contributes to correct diagnosis. On the other hand, it is only rarely applied in transplant kidney pathology unless recurrence of the native kidney disease is suspected.

The primary cause of kidney allograft loss is still chronic rejection, followed by death with a functioning allograft and primary kidney disease recurrence. Kidney allograft rejection can be classified into two types - T cell- and antibody-mediated rejection (AMR) representing two out of six categories from the Banff 2013 classification of allograft pathology. EM can provide useful information for AMR diagnosis, but also in BK-virus nephropathy, recurrent disease, and de novo glomerulopathy.

The characteristic light microscopy histopathological features of AMR include microvascular inflammation, presenting as glomerulitis and peritubulitis with or without positive staining for the C4d component of the complement. However, alterations at the cellular level, such as changes in cytoskeleton and cell morphology, as well as matrix synthesis can only be detected with EM. The distinctive AMR features seen with EM in the glomeruli include double contours of capillary walls associated with

endothelial widening (cg1a) and in tubulointerstitium, peritubular capillary basement membrane multilayering (PTCML). The combination of EM glomerular endothelial cell swelling, subendothelial electron-lucent widening, and early glomerular basement duplication, with or without C4d positivity, is strongly associated with AMR. These EM-only features precede double contours visible by light microscopy and their timely treatment might attenuate the progression of transplant glomerulopathy (TG) associated with AMR. Progression from EM-only cg1a to more severe cg may be a useful surrogate endpoint when developing novel treatments for AMR.

The EM presentation of PTCML is characterized by a growing number of peritubular capillaries basement membrane layers. Studies have confirmed a strong association between the presence of high-level PTCML and AMR, but low-level PTCML should not be overlooked as it precedes chronic AMR. The current threshold for establishing

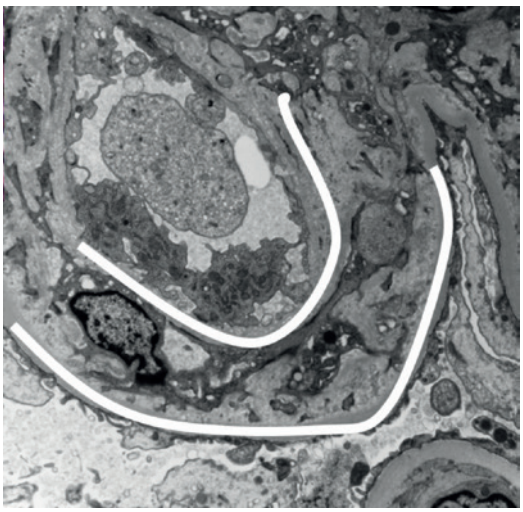


Figure 2.
Ultrastructural changes in a glomerular endothelial cell in AMR (multiplication of basement membrane marked with white lines)

The EM presentation of PTCML is characterized by a growing number of peritubular capillaries basement membrane layers. Studies have confirmed a strong association between the presence of high-level PTCML and AMR, but low-level PTCML should not be overlooked as it precedes chronic AMR. The current threshold for establishing chronic active AMR is at least one peritubular capillary with ≥7 basement membrane layers or at least two with ≥5 layers. However, in transplanted patients with donor-specific HLA antibodies, the presence of higher levels of PTCML (>2.5 layers) in a biopsy specimen around one-year post-transplantation is predictive of future TG.

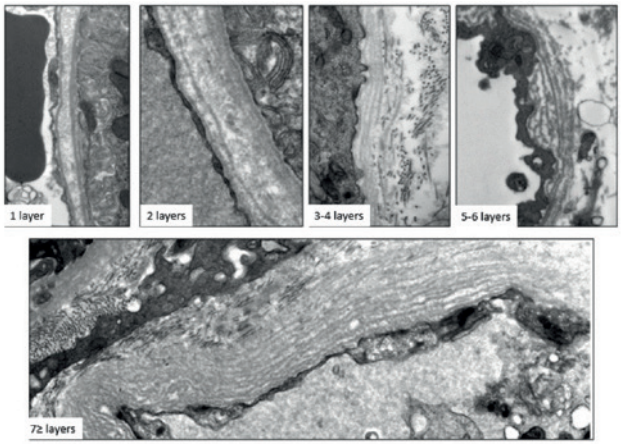


Figure 3.
Peritubular capillary membrane multi lamination in AMR

MicroRNA and chemokine profiles in urine to identify renal transplant rejection

Michael Eikmans, Netherlands

Around 10-15% of kidney transplant patients develop acute rejection (AR). The main histopathological patterns of AR include mononuclear cell infiltrate, tubulitis, and sometimes even vasculitis. AR is commonly diagnosed based on the sudden decline in estimated glomerular filtration rate, and graft biopsy is the gold standard for the diagnosis. However, the major limitations of this procedure are invasiveness, sampling errors, and inter-individual variations. Thus, there is an unmet need for noninvasive tools for the timely diagnosis of AR.

A recent study explored an integrated approach to diagnosing AR consisting of a combination of serum creatinine, graft biopsy, and urine biomarkers. Urine samples were taken at transplantation and centrifuged to determine the levels of CXCL-9 and CXCL-10. Both CXCL-9 and CXCL-10 are inflammatory chemokines, previously shown to be actively involved in AR. Urine sediment was also analyzed for microRNAs (miRNA). MiRNAs are small, highly conserved non-coding RNA molecules involved in the regulation of gene expression. Due to their prolonged stability compared to standard RNA, miRNAs represent a compelling target for biomarker research. The pilot study for miRNA screening included 31 patients transplanted between 2007 and 2015, of whom 15 had AR. This was followed by a validation study on another 140 patients transplanted in the same period, of whom 90 had AR. In the pilot study, several miRNAs exhibited higher or lower levels in patients with AMR compared to the other group. In the validation study, a total of 15 miRNAs were tested in urine and some of them showed different expression between the AR and non-AR groups. Specifically, miRNA-155-5p exhibited significantly higher, while miRNA-615-3p exhibited significantly lower levels in patients with AR. Both CXCL-9 and CXCL-10 levels were significantly higher in the AR cohort. Further multivariate analysis identified a combination of two miRNAs and CXCL-9, with or without recipient age to have the highest sensitivity and specificity in predicting AR. Further cross-validation tested the model's ability to predict new data that was not used in the initial estimation. The stratified 10-fold cross-validation showed similar results as the initial model, thus confirming its reliability.

The study concluded that the combination of urinary miRNA- and chemokine profiles successfully distinguishes kidney transplant rejection from stable transplant conditions. Further investigation should address the adequate frequency of transplant biopsies to timely detect rejection episode and define how much in advance a particular analyte predict the first signs of rejection. The specificity of biomarkers in distinguishing rejection from infection should also be evaluated.

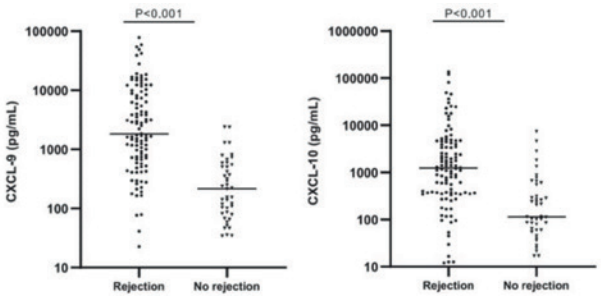


Figure 4.
Urine levels of CXCL-9 and CXCL-10 in transplanted patients with and without AR (from ref. 15)

Further readings

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Written by Jasna Trbojevic-Stankovic.
All the speakers reviewed and approved the content.