Commentary

Are we ready to implement non-invasive tests to detect allograft rejection in a daily praxis?

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In this article of *EBioMedicine*, Sofia Christakoundi and colleagues report on results of KALIBRE study. In this study on 248 patients, authors prospectively evaluated 20 peripheral blood derived transcripts and further validated a panel of 7 genes which predict the occurrence of T cell mediated rejection seven weeks ahead of kidney graft biopsy confirmers rejection diagnosis. Authors thus suggest peripheral molecular marker alterations may reveal subclinical intrarenal alloimmune processes and suggest further clinical testing. Moreover, they were able to detect another set of six-genes which were predictive for polyomavirus BK infection [1].

A development of a reliable non-invasive diagnostic test capable distinguishing early stages in the development of rejection is clearly the unmet medical need in organ transplantation [2]. In kidney transplantation, majority of grafts are being lost due to graft rejection which is caused by donor specific anti HLA antibodies and may occur at any time. Contrary, T cell mediated rejection occurs within the first several weeks or months and responds well to given antirejection therapy. Although most of T cell mediated rejections has a benign phenotype, its early non-invasive detection may prevent future graft biopsy and higher hospitalization costs.

The monitoring of serum creatinine or eGFR has poorer predictive value to detect ongoing active rejection as there are many other reasons for its increase. The increase of urine concentration of several tubular proteins such as neutrophil gelatinase-associated lipocalin (NGAL) or kidney injury molecule-1 (KIM-1) early after transplantation reflect more ischemic than alloimmune graft injury. Similarly, the increase of urine concentration of interferon gamma-induced protein 10 (IP-10) lacks specificity to distinguish rejection from other causes of acute kidney injury. Therefore, it seems that evaluation of mRNA transcripts associated with alloimmune response might be more specific for rejection.

Suthanthiran's group evaluated several transcripts in urine sediments in kidney transplant recipients, and found three-gene signature (CD3ε mRNA, IP-10 mRNA, and 18S rRNA) to be diagnostic and prognostic of acute cellular rejection in kidney allografts [3]. Of note, these transcripts were increased up to 20 days before biopsy-proven rejection occurrence. However, those data have not been replicated by others, mainly because of complexity of the method to isolate RNA from fresh urine derived cells.

Authors of KALIBRE study were thus among first who analysed peripheral blood transcripts in renal transplant recipients in Europe. Recently, the Assessment of Acute Rejection in Renal Transplantation (AART) study used a 17-gene set (the Kidney Solid Organ Response Test - kSORT) for prediction of acute rejection. Ten of those transcripts were shown to play a role in acute rejection while the others were described to be associated with activated monocytes, endothelial cells and T cells. kSORT was able to detect AR in blood independent of age, time post-transplantation, and sample source without additional data normalization as early as 3 months before histological diagnosis [4]. In the both of Roedder's and Christakoundi's studies authors selected studied genes based on previous intragraft pathways and networks analyses. Clearly, simplification of this selection may be biased in comparison to microarray-based approach and several more-predictive genes could be missed. Moreover, peripheral blood transcriptome reflects more complex environment where immune responses to pathogens and autoantigens cannot be differentiated from alloimmune responses. Of note, in Christakoundi's study a good half of healthy controls had present molecular signature of T cell mediated rejection in peripheral blood. Both works also differed in the used induction immunosuppression. While in the US cohort patients received T cell depletive induction, in the European one no depletive agent was used in the discovery set. Therefore, it remains unclear whether KALIBRE study results can be generalized to other cohorts.

Contrary to both, a previous prospective single center study aimed to monitor peripheral transcripts associated with hyporesponsiveness to grafted tissue. Set of genes was selected from microarray studies on operationally tolerant patients whose were off the immunosuppression but have no signs of destructive alloimmune response [5]. The study showed that patients on triple immunosuppression with higher peripheral transcripts associated with immature B cell function (operational tolerance markers) experienced less rejections within the first posttransplant year [6].

Finally, a study on circulating donor-derived cell-free DNA (dd-cfDNA) in blood for diagnosing acute rejection in kidney transplant recipients (DART) validates that plasma levels of dd-cfDNA can discriminate active rejection status. Plasma levels of dd-cfDNA were measured in a cohort of 102 patients and correlated with allograft rejection. The study showed that dd-cfDNA level discriminates between biopsy specimens showing any rejection (T cell mediated or antibody mediated rejection) and rejection-free controls (receiver operating characteristic

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area under the curve 0.74) and dd-cfDNA levels >1% indicated a probability of active rejection [7]. However, in another recent study dd-cfDNA test did not discriminate cellular rejection from no rejection among kidney transplant recipients, although performance characteristics were stronger for the discrimination of antibody mediated rejection [8]. Other studies with more optimized tests [9] thus need to be performed, but at this moment it seems that dd-cfDNA test is useful for monitoring at least in sensitized patients with present donor specific anti HLA antibodies.

In kidney transplantation, there are no biomarkers used in a daily routine which measure the status of alloimmune injury. Christakoundi and colleagues from KALIBRE study need to be applauded for their great effort to define set of non-invasive molecular markers of acute rejection in kidney transplantation. However, theirs and others' results still need to be considered preliminary and more studies with simplified molecular tests useful for a daily routine on several larger and independent cohorts are warranted.

Authors contribution

O.V. wrote the manuscript.

Conflict of interest

The author declares no conflict of interest.

Acknowledgments

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