Response to: "Is kSORT adequate to detect renal transplant patients at high risk of acute rejection?"

Respuesta al artículo: "¿El test kSORT es adecuado para detectar a los pacientes trasplantados renales con riesgo alto de rechazo agudo?"

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We would like to respond to the article written by Raoch et al[1] on "The kSORT assay to detect renal transplant patients at high risk for acute rejection: results of the multicenter AART study".[2] We appreciate the authors' recognition of the kSORT assay and acknowledge the complexity of the AART study; we would like to take this opportunity to address the concerns raised by the authors, particularly regarding study design and kSORT development:

The AART (Assessment of Acute Rejection in Renal Transplantation) study was intentionally designed to include heterogeneous patients and samples representing the "real-life" post-transplant setting which, rather than being a weakness of the study design, is a clear strength, as it allowed for the development of kSORT to robustly detect acute rejection (AR) based on gene expression in peripheral blood. Previous studies have clearly demonstrated the influence of peripheral blood sample processing methodologies on gene expression;[3,4] and kSORT demonstrated equal performance in peripheral blood samples collected according to three different processing methodologies, a key result in the AART study significantly strengthening the assay.[2]

As described by the authors in the original article, AART included 558 independent peripheral blood samples collected from 436 patients (116 pediatric and 320 adult renal transplant recipients). The 95 pediatric patients from Stanford University were enrolled in an investigator-initiated clinical trial[5] with protocol biopsies performed in addition to clinically indicated ones; all other patients including 21 pediatric patients from Mexico were part of the regular patient management program of the transplant centers that performed biopsies at clinically indicated time-points; Barcelona and Mexico additionally performed protocol biopsies. Except for AART191 which included serially collected samples, AART143, AART124, and AART100 included cross-sectional samples. All samples in AART were independent and were collected within 48 h of a renal allograft biopsy and before any treatment intensification. As illustrated in Figure 1 of the original article,[2] 58 out of the total 436 patients in AART provided multiple samples such that AART191 included samples from 19 patients who also provided samples to AART143, and included samples from 18 patients who were also included in AART124. Similarly, AART100 included samples from 21 patients who also provided samples to AART143. The definite inclusion and exclusion criteria for samples, as well as for AR and No-AR class allocation are stated in the original article.[2] Importantly, while we agree with Raoch et al. that the missing centralized histological evaluation of the biopsies was a limitation of the AART study, it has to be also taken into account that all samples were scored using the same criteria,[6] the most standardised histological classification to grade transplant allografts; thus, the potential bias inferred was homogenously distributed in all histological evaluations. Indeed, we would like to point out that this was stated in the original article.

As correctly stated by Raoch et al., the kSORT assay was developed in several stages. We would like to clarify the development of the kSORT algorithm, kSAS: kSAS is a correlation-based algorithm that correlates gene expression profiles in unknown samples to the corresponding gene expression profiles in known AR- and known No AR-reference profiles. kSAS was developed in AART100 and consists of 13 different gene models, each utilizing 12 of the 17 kSORT genes. The kSAS algorithm was initially validated in AART143 applying a single model of 14 of the 17 kSORT genes.

Finally, and as stated in the paper, the kSORT assay needs to be prospectively validated in additional independent cohorts of kidney transplant patients. To date, kSORT has been further evaluated in two additional independent clinical studies, confirming the results obtained in AART with proven high sensitivity and specificity and excellent negative and positive predictive values.[7,8] The interim results of kSORT in the SAILOR study, a randomized multicenter trial of 222 renal transplant recipients,[8] confirmed that kSORT has 93.3% sensitivity and 90% specificity and 98.6% negative predictive value for the diagnosis of AR and is not confounded by BK viremia. Furthermore, 73% of AR could have been diagnosed by kSORT prior to their current time-line for diagnosis based on serum creatinine alone.[9] In the ESCAPE study,[10] kSORT was evaluated together with the functional IFN-γ ELISPOT assay to detect subclinical AR. kSORT showed high accuracy in predicting subclinical AR (98% specificity, 93% positive predictive value), and also correlated with histological Banff scores. The kSORT results in ESCAPE further supported the application of the assay to monitor the efficacy of rejection treatment. Combining kSORT with the IFN-γ ELISPOT increased the prediction precision and additionally allowed identification of the main immune effector mechanisms of rejection.[10]

While the clinical utility of kSORT is currently being established in ongoing interventional clinical trials, ImmucorDX an independent certified clinical laboratory (CLIA# 23D1054909; Grand Rapids, MI), has made kSORT available as a laboratory developed test (LDT) for renal transplant patient immune surveillance in conjunction with the current standards of care. The analytical accuracy, precision, sensitivity and specificity of kSORT were validated by ImmucorDx. Precision analysis demonstrated an average result agreement of 94% across six samples tested over five days, and showed non-interference of kSORT with creatinine, bilirubin, triglycerides, tacrolimus, prednisone, and IVIg. Analytical accuracy analyses demonstrated that kSORT yielded an 11% indeterminate rate, 100% sensitivity, and 96% specificity for the detection of biopsy-proven AR, excluding indeterminate calls. These findings are consistent with the findings of AART, ESCAPE, and SAILOR.

Competing interests: SR and TR are employees of Immucor.

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