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Calcineurin Inhibitors and Variation in the Performance of Interferon-γ Release Assays Used to Detect Tuberculosis Infection

To the Editor:

A key strategy of tuberculosis (TB) control programs in high-resource countries is identification of latent tuberculosis infection (LTBI) and preventive therapy to avert progression to TB disease (1). Currently, only tuberculin skin tests (TSTs) and interferon-γ release assays (IGRAs) are used for LTBI screening (2). IGRAs are functional blood-based assays that detect interferon-γ produced by memory T cells after stimulation with mycobacterial antigens (2). Currently, two IGRAs are available: the T-SPOT.*TB* assay (Oxford Immunotec) and the more widely used QuantiFERON-TB Gold (QFT) assay (Cellestis/Qiagen) (3).

Globally, the number of hematopoietic stem cell transplant and solid organ transplant recipients is rising steadily. Transplant recipients require long-term immunosuppression and consequently have a much greater risk of developing TB disease than the general population (4). Furthermore, mortality associated with TB disease is higher (4–6). Calcineurin inhibitors, including cyclosporin and tacrolimus, are the most commonly used immunosuppressive agents after transplant (7). They reduce T cell activation, thereby inhibiting production of various cytokines, including interferon-γ and interleukin 2 (IL-2) (8). Both cytokines play crucial roles in human antimycobacterial immune responses (9, 10).

TB screening in patients receiving immunosuppressive medication is complex (4, 11–13). Considerable evidence shows that the sensitivity of TSTs is reduced in immunocompromised individuals (2, 14). Previous studies investigating IGRAs in the transplant setting have reported conflicting results, some suggesting that they are reliable and others concluding that their performance is impaired (15–18). The key limitation of all previous clinical studies is that no gold standard for LTBI exists (2). Therefore, the interpretation of negative IGRA results in immunosuppressed patients is difficult, because it is currently impossible to distinguish true absence of TB infection from a false-negative result caused by immunosuppression.

The aim of this study was to determine the impact of calcineurin inhibitors on the performance of QFT assays using an *ex vivo* model. In addition, we investigated their impact on recently identified biomarkers of TB infection: mycobacteria-specific IL-2, interferon-γ–inducible protein 10 (IP-10), and tumor necrosis factor-α (TNF-α) responses (9, 10).

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Methods

Study population. Adults with a previous positive IGRA result or recent TB exposure were recruited at a TB clinic after written informed consent was obtained. Potential participants with known immunodeficiency or receiving immunosuppressive medication were excluded. The study was approved by the National Research Ethics Service Committee (13/SC/0043).

Interferon-γ release assays. From each participant, three sets of QFT assays comprising an antigen-stimulated, a positive (mitogen) control, and a negative control tube were obtained. No reagents were added to the first set ("standard assay"). In the second set, cyclosporin (Sandimmune; Novartis) was added to each tube to a final concentration of 200 ng/ml, a common target level in the hematopoietic stem cell transplant setting (19). In the third set, tacrolimus (Prograf; Astellas Pharma) was added to each tube to a final concentration of 10 ng/ml, a typical target level in the solid organ transplant setting (20). Drugs were added within 4 hours of phlebotomy, and samples were immediately transferred into a 37°C incubator. After 24 hours, supernatants were harvested as per the manufacturer's instructions, followed by cryopreservation.

Cytokine measurements. Cytokine concentrations in supernatants were determined with ProcartaPlex xMAP assays (Affymetrix/eBioscience) measuring interferon-γ, IP-10, IL-2, and TNF-α according to the manufacturer's instructions. Their broad dynamic range allows accurate measurement of the high interferon-γ concentrations that often occur in QFT assays, which exceed the upper limit of QFT enzyme-linked immunosorbent assays (13). Assays were read with a Luminex 100 Bioanalyzer with xPONENT software (Luminex Corporation).

Interpretation of QFT results. QFT results were interpreted according to the latest version of the manufacturer's package insert (U.K. version). Briefly, a positive result was defined as a background-corrected interferon-γ response $\geq$ 0.35 IU/ml and simultaneously $\geq$ 25% of the nil control sample interferon-γ concentration. A negative result was defined as a response below this threshold in the presence of a valid positive control (i.e., background-corrected interferon-γ concentration $\geq$0.5 IU/ml). An indeterminate assay result was defined as a sample set in which the negative control failed (i.e., interferon-γ concentration >8.0 IU/ml) or in which the positive control failed (background-corrected interferon-γ concentration <0.5 IU/ml).

Statistical analyses. All cytokines were analyzed in picograms per milliliter, except interferon-γ, which was measured in

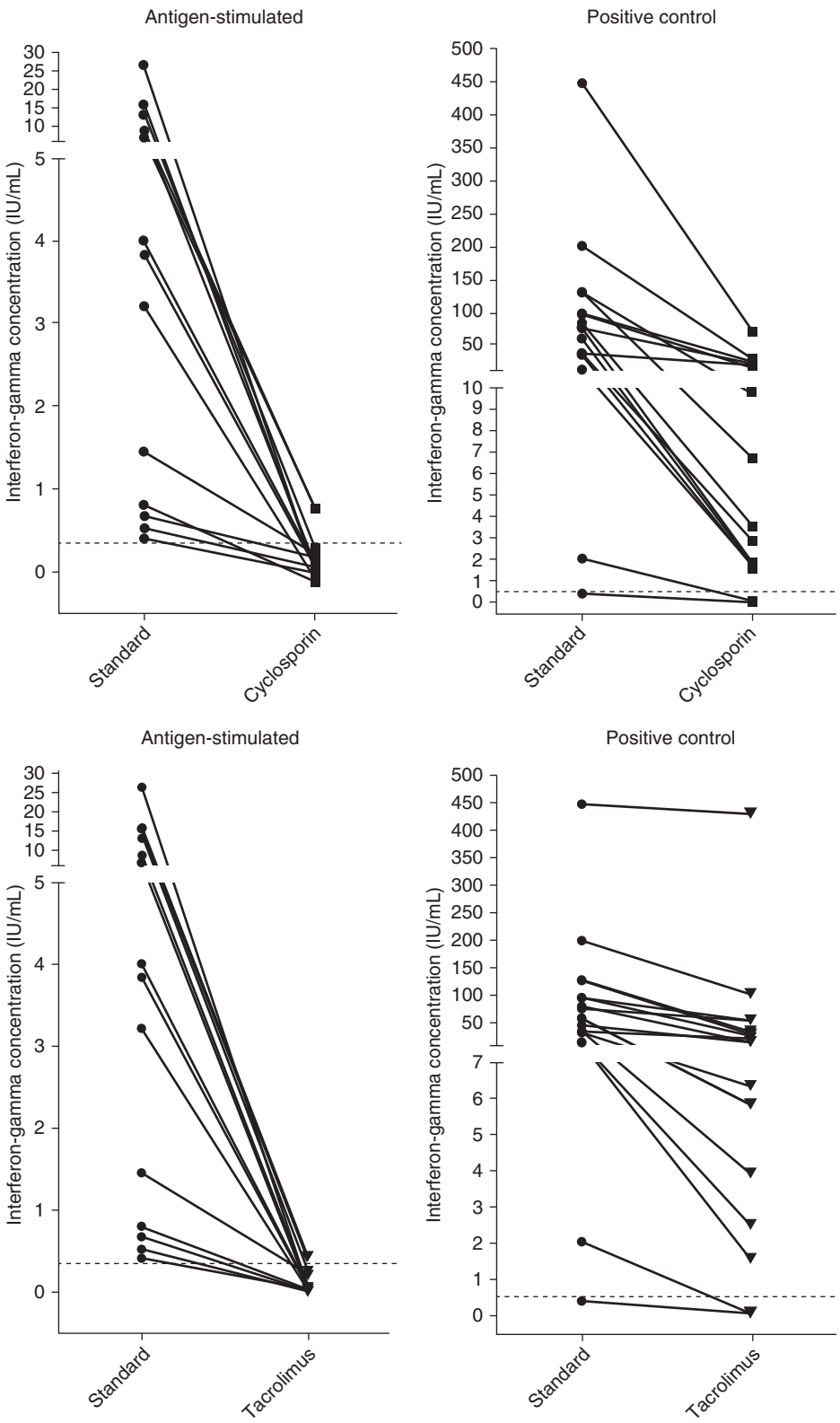

Figure 1. Background-corrected interferon-γ concentrations in antigen-stimulated (left) and positive control (right) samples in individual participants in the standard assay set compared with sets with added cyclosporin (upper panel; *n* = 13) and tacrolimus (lower panel; *n* = 13). Dotted lines indicate the cutoff for a positive test result in antigen-stimulated samples (0.35 IU/ml) and the cutoff for a valid positive control response (0.5 IU/ml).

picograms per milliliter and then converted to international units per milliliter (the units used in QFT assays) for analysis, as previously described (21). Statistical comparisons were done in Prism software (version 6.0; GraphPad Software) using Wilcoxon matched-pairs signed-rank tests.

Results

A total of 18 participants were recruited, of whom 13 had positive QFT results. For the analyses of antigen-stimulated cytokine responses, only data from these 13 participants were included, whereas for the analyses of positive control responses, data from all 18 were included.

Interferon-γ responses and categorical QFT results. Both cyclosporin and tacrolimus caused considerable reductions in background-corrected interferon-γ concentrations in the antigen-stimulated samples in all participants (Figure 1). Compared with the standard assay (3.84 IU/ml; interquartile range [IQR], 0.74–10.9), the median interferon-γ concentrations were significantly lower in the cyclosporin- and tacrolimus-treated assay sets (0.0 IU/ml; IQR, −0.12 to 0.18; $P < 0.001$; and 0.02 IU/ml; IQR, −0.006 to 0.13; $P < 0.001$, respectively) (Figure 2A). In the cyclosporin- and tacrolimus-treated positive control samples, the median interferon-γ concentrations were also significantly lower (5.1 IU/ml; IQR, 1.6–18.9; and 14.3 IU/ml; IQR, 3.5–39.1, respectively)

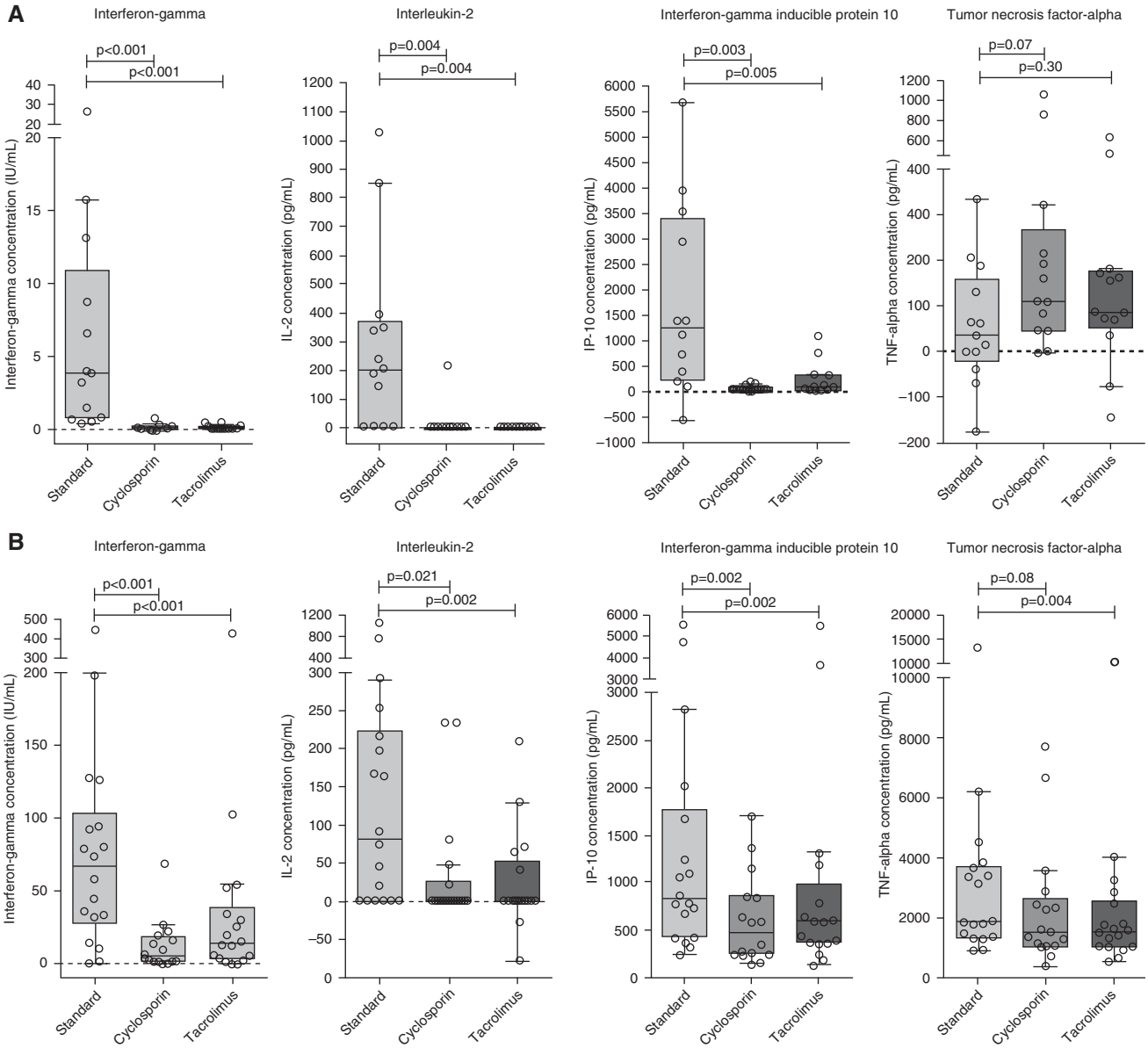

Figure 2. Background-corrected interferon-γ, interleukin 2 (IL-2), interferon-γ–inducible protein 10 (IP-10), and tumor necrosis factor-α (TNF-α) concentrations in (*A*) antigen-stimulated ($n = 13$) and (*B*) positive control ($n = 18$) samples in standard assay sets and sets with added cyclosporin and tacrolimus. Box plot with Tukey whiskers; horizontal lines depict the medians; *P* values were calculated with Wilcoxon matched-pairs signed-rank tests. Negative values are due to background correction (*see* the METHODS section).

than in the standard assays (66.6 IU/ml; IQR, 28.0–103.3) but still considerably above the cutoff for classifying positive control samples as failed (Figure 2B).

Of the 13 participants with a positive QFT result in the standard assay, 10 converted to a negative result in the cyclosporin-treated set and 2 to an indeterminate result, and 1 (participant 4) continued to have a positive result despite a markedly reduced antigen-stimulated interferon-γ response (0.76 vs. 6.59 IU/ml in the standard assay). In the tacrolimus-treated set, 10 individuals converted to a negative and 2 to an indeterminate result, and 1 (participant 1) remained positive, again with markedly reduced response (0.43 vs. 13.1 IU/ml).

IL-2, IP-10, and TNF-α responses. Background-corrected IL-2 and IP-10 concentrations were significantly lower in the antigen-stimulated samples in the cyclosporin- and tacrolimus-treated assay sets than in the standard assay (Figure 2A). In contrast, there was no significant difference in background-corrected TNF-α concentrations. TNF-α responses in the positive control samples were also largely maintained, although statistically there was a significant reduction in concentrations in tacrolimus-treated samples (Figure 2B).

Discussion

This study provides robust evidence that calcineurin inhibitors have a significant adverse effect on the performance of IGRAs. Our results suggest that the majority of patients with LTBI who are receiving treatment with cyclosporin or tacrolimus would have false-negative IGRA results when screened for TB, such as in the context of contact screening after exposure to a case with pulmonary TB. Importantly, the *ex vivo* model used in this study cannot capture the long-term impact of calcineurin inhibitors on T cells, which may be even more pronounced.

The marked impact of calcineurin inhibitors on IGRAs is consistent with their known mechanism of action. A key property of this drug class is inhibition of T cell activation and suppression of proinflammatory cytokines, including interferon-γ and IL-2, in T cells (8, 22, 23), the main source of interferon-γ in functional assays determining antimycobacterial immune responses, including QFT assays (2). The observed reduction in IP-10 responses is also predicted, because IP-10 production is primarily induced by interferon-γ (24). It is unlikely that those observations are due to cytotoxicity, because previous data show that even at a 100-fold greater concentration than used in this study cyclosporin has no significant cytotoxic effects on T cells (25).

In contrast, TB antigen–induced TNF-α responses were not suppressed by cyclosporin or tacrolimus. This suggests that calcineurin inhibitors have only limited effect on macrophages, the principal source of TNF-α in immune responses directed against mycobacteria, consistent with published data (26). Furthermore, this observation suggests that in patients receiving calcineurin inhibitors, novel TB assays based on TNF-α responses, which are currently in development (9, 10), may prove more robust than IGRAs.

In conclusion, considering our results together with previous data showing that the performance of TSTs is also impaired in immunosuppressed patients, both currently used LTBI screening tests should be regarded as unreliable in patients receiving calcineurin inhibitors. Although a positive IGRA result remains useful in this patient population, a negative result provides no meaningful information regarding TB infection status.

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Prehospital Emergency Care in Sepsis: From the "Door-to-Antibiotic" to the "Antibiotic-at-Door" Concept?

To the Editor:

In the December 2018 issue of *AnnalsATS*, Peltan and colleagues reported that for patients with sepsis without hypotension, antibiotic initiation is faster when patients are cared for by a prehospital advanced life support team, but not a basic life support team (1). Although the authors did not report the effect on a strong outcome parameter (i.e., mortality), their results promote systematic care of patients presenting with sepsis symptoms by an advanced life support team.

Nevertheless, as underlined by the authors (1), for sepsis, long antibiotic delays are associated with poorer outcomes. To date, no results are available from randomized controlled trials to determine the effect of prehospital antibiotic administration for patients presenting with sepsis (2). Unfortunately, previous studies that evaluated this strategy have shown negative results (3), but this could be at least partly explained because most of these trials have recruited patients with varying levels of septic severity, and not only those presenting with septic shock (4). Furthermore, from an emergency medical service point of view, the criteria proposed by the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) do not seem to be appropriate (3). Indeed, excluding the most caricatural septic cases, early identification of the sepsis and assessment of its severity during the phone call to the emergency medical service dispatch center are difficult (4), but conversely, it represents the prerequisite needed to determine the appropriate care response (advanced life support vs. basic life support) for an individual patient.

Finally, beyond early sepsis recognition, functional and survival prognosis of patients could be much more improved not only after an isolated specific intervention such as prehospital antibiotic administration (5), but also after introduction of a "bundle of care" strategy, including hemodynamic optimization. To date, the SAMU Save Sepsis is the only trial that evaluates the effect of prehospital initiation of a bundle-of-care strategy on mortality in severely septic patients (6). This French prospective multicentric study aims to determine whether an aggressive therapeutic option, with early antibiotic administration, fluid loading, and eventually catecholamine administration, initiated early "at the door" of the patient by a prehospital medical emergency medical service team, could allow for a reduction in the mortality of patients suffering from severe sepsis and/or septic shock.

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