

O138

2-hour Oral Session

Fungal diagnostics

### Cyclosporin A, MMF, and prednisolone lead to reduced sensitivity of the quantification of *Aspergillus*-specific T-cells

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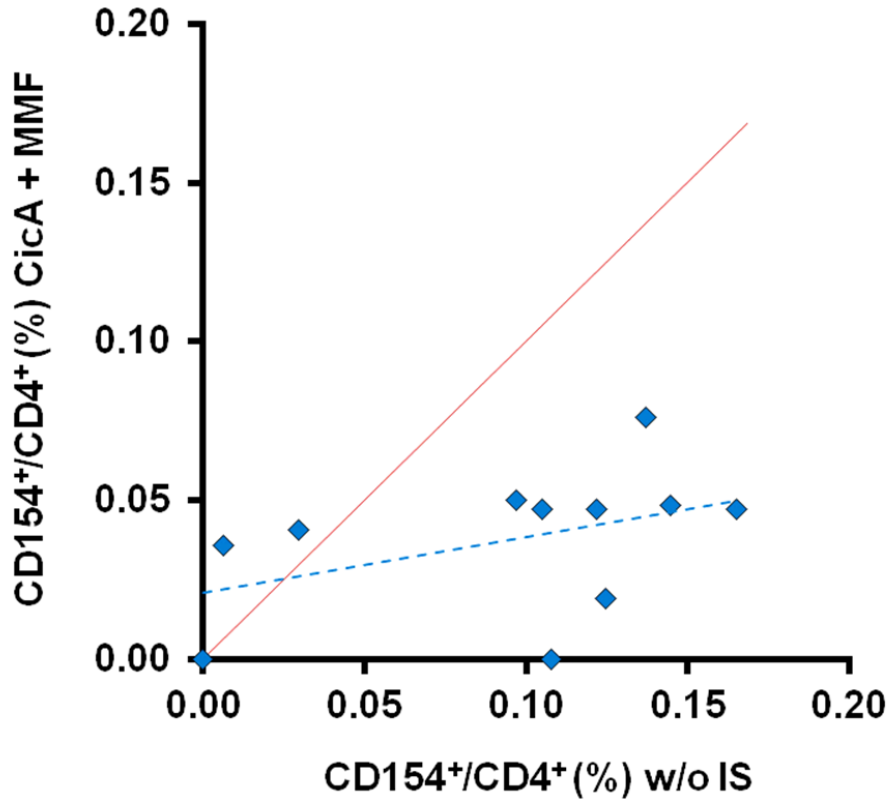
**Background:** *Aspergillus*-specific T-cells were described as a novel biomarker for early diagnosis of invasive aspergillosis (IA) in patients undergoing hematological stem cells transplant (HSCT). These patients receive T-cell suppressive GvHD prophylaxis regimes often containing ciclosporin A and/or mycophenolate-mofetil (MMF). Corticosteroids are commonly used for the initial therapy of acute GvHD. This study sought to determine, whether ciclosporin A, MMF, and prednisone influence the sensitivity of the quantification of *A. fumigatus* specific T-cells.

**Material/methods:** 54 ml of heparinized blood were collected from 11 healthy adult donors. PBMCs were isolated and plated into a 6 Well-plate at a concentration of  $5 \times 10^6$  cells/ml. Mycophenolate-mofetil (2 µg/ml), ciclosporin A (200 ng/ml), and/or prednisolone (200 ng/ml) were added. Cells were incubated at 37 °C, 5% CO<sub>2</sub> for 3 h. Subsequently, the cells were harvested and resuspended in RPMI 1640 supplemented with 5% autologous serum at a concentration of  $1 \times 10^7$  cells/ml. 100µl of the cell suspension were plated into a 96 well-plate. Cells were stimulated with a commercially available *A. fumigatus* mycelial lysate, treated with Brefeldin A, and stained as previously described. The percentage of CD154<sup>+</sup> cells among CD4<sup>+</sup> cells was quantified by flow cytometry.

**Results:** In absence of immunosuppressive drugs a mean frequency of 0.10 % ± 0.05 % *A. fumigatus* specific T-cells was observed. If PBMCs were pre-treated with therapeutic concentrations of mycophenolate-mofetil and ciclosporin A for 3 h, the mean frequency declined to 0.04 % ± 0.02 (p < 0.01, Figure A). Sole exposure of PBMCs to mycophenolate-mofetil (0.05 %, p = 0.02) or ciclosporin A (0.03 %, p = 0.02) was also associated with a significantly decreased mean frequency of specific T-cells (Figure B). Combined pre-treatment of the cells with prednisolone plus MMF resulted in a further reduction of assay sensitivity (0.01%, p = 0.03).

**Conclusions:** These findings demonstrate that the quantification of *A. fumigatus* specific T-cells is significantly impaired by cell exposure to therapeutic concentrations of T-cell suppressive agents. Hence, test results should be interpreted carefully when using the assay in HSCT patients receiving GvHD prophylaxis or therapy. In particular, false-negative test results need to be considered in these patients.



**A****B**