P3042

Development of a novel standardized and fully automated functional assay to assess and monitor global T cell immune function in 4 hours

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Abstract

Background

Immunosuppressed patients with impaired cell-mediated immunity have a higher risk of secondary infection contributing to clinical worsening and increased mortality. In routine practice, simple, rapid and standardized immune assays to functionally assess the individual level of immunosuppression are clearly missing. For instance, in solid-organ transplant patients, monitoring of immunosuppressive magnitude mainly relies on drug concentration measurements not known to be sufficiently accurate. Consequently, these approaches are often not precisely tailored to each individual patient's needs. Assessing global individual T cell functionality is therefore paramount. This study aims to describe the potential and clinical relevance of a novel functional assay on fully automated VIDAS® instrument to answer the unmet needs in assessing T cell functionality for routine care.

Methods

Freshly blood was collected in lithium heparin tubes from different cohort of immunosuppressed patients and healthy volunteers. Functional immune response was then assessed in 4 hours by our automated and standardized VIDAS® STIMM™ T RUO, an assay in which cytokine released by T cells from whole blood was measured after stimulation. Peripheral blood count was also performed to assess T lymphocyte subsets (CD3, CD4, CD8) by flow cytometry.

Results

T cell functional immune response was assessed by the VIDAS® STIMM™ T RUO in 11 healthy volunteers, 20 patients after liver transplantation (sequential sampling after transplantation) and 22 septic shock patients (sequential samples after ICU admission). We observed that VIDAS® STIMM™ T RUO allowed to stratify patients into low, medium and high T cell immune response subgroups, independently of the total T cell number. We assessed also the correlation with the patients' clinical course.

Conclusion

VIDAS® STIMM™ T RUO embodies the next generation of assays capable of assessing and monitoring global T cell functionality in a rapid, simple, fully automated and standardized way. It may help clinicians for routine care and clinical decision-making.

Methodology

Design of a standardized and fully automated IGRA to monitor antigen-independent T cell functionality

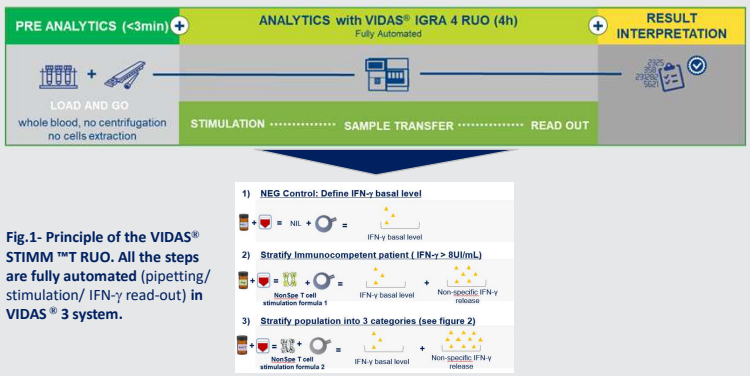


Fig.1- Principle of the VIDAS® STIMM ™T RUO. All the steps are fully automated (pipetting/ stimulation/ IFN-γ read-out) **in VIDAS ® 3 system.**

Definition of the VIDAS® STIMM™ T RUO cut-offs

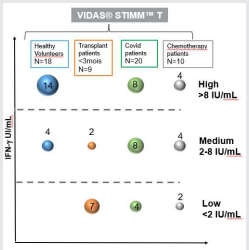


Fig.2- Preliminary data of **VIDAS® STIMM ™ T RUO with immunosuppressed populations allow to stratify patient's T cell functionality into low, moderate, and high** by placing 2 cut-offs of < 2 IU/ml and > 8 IU/ml

Results

Assessment of T cell functionality in Liver transplantation (LT) patients with VIDAS® STIMM ™ T RUO

Study design

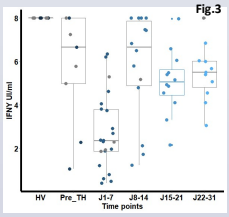
11 healthy volunteers (HV) and **20 LT patients** (EdMonHG study) were enrolled. T cell functionality from whole blood of LT patients was assessed before and at 1, 2, 3, 4 weeks following transplant by the VIDAS® STIMM ™ T

Assessment of LT patient T cell functionality with VIDAS® STIMM™T RUO - Fig.3.

- Starting before transplantation, VIDAS® STIMM™ T RUO values (IFN-γ levels after stimulation) were significantly lower in LT patients compared to HV during the month of follow-up (including pre-transplant). Results between patients are quite homogeneous at W3 and 4 despite the fact that some patients have high T cell functionality (IFN-γ> 8UI/mL)

- Correlation between T cell functional immune response assessed by VIDAS® STIMM™ T RUO and the occurrence of clinical events reflecting post LT immunodysfunction will be analyzed at the end of the EdMonHG study.

Fig.3



Assessment and monitoring of T cell functionality in septic shock patients with VIDAS® STIMM™ T RUO

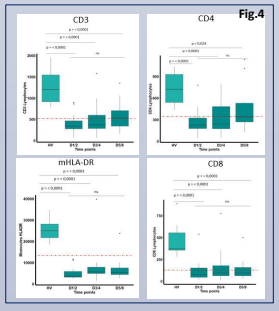
Study design and population

A total of 11 healthy volunteers (HV) and **22 septic shock (SS) patients** (Immunosepsis study) were enrolled (Tab.1). Blood was analyzed from SS patient at D1-2 (N=19), D3-4 (N=19), D5-8 (N=14) days of follow up. White blood cell counts and assessment of monocyte HLA- DR (mHLA-DR) were conduced by cytometry (Fig.4). T cell functionality was assessed by the VIDAS® STIMM ™ T (Fig.6) on VIDAS ® 3 system.

Gender_ n (%)	14 (63,6)
Age _ median (IQR)	72 (63 – 76)
SOFA_ median (IQR	9 (7-10)
SAPSII_ median (IQR)	57 (51 -63)
Charlson_ median (IQR	2 (0 – 3)
Primary site of infection	
Respiratory _ n (%)	6 (27,3)
Intra abdominal _ n (%)	11 (50)
Urinary _ n (%)	3 (13,6)
Adverse Outcome	
Nosocomial Infection _ n (%)	4 (18,2)
28-day mortality _ n (%)	4 (18,2)

Table 1- Clinical patients characteristics and outcomes

Fig.4



Analysis of SS patient T lymphocyte subsets (CD3, CD4, CD8) and mHLA DR expression by Flow Cytometry- Fig.4

-SS patients had a **significant decrease of total T lymphocyte count including CD4 and CD8 T** lymphocytes, as well as a **diminished expression of mHLA-DR** compared to the HV, on the first day of ICU admission and during the first week.

- In survivors, CD3+ and CD4+ counts initiate a slightly increase after 1 week, while CD8+ count and mHLA DR expression remain constantly decreased.

Assessment of SS patient T cell functionality with VIDAS® STIMM™ T RUO – Fig.5

-T cell functionality of SS patients can be categorized as low (IFN-γ < 2IU/ml), moderate (2<IFN-γ >8 IU/ml) or high (IFN-γ > 8 IU/ml) compared to HV (IFN-γ > 8 IU/ml)

-**On the first day of ICU admission, T cell functionality of SS patients appears predominantly low (group IFN-γ < 2IU/ml in 53% of the patients)** compared to D3/4 and D5/8. Interestingly, there is no significant correlation between the level of IFNγ and the total lymphocyte counts (**Fig.6**) or T cell subset CD4+ (**Fig.7**). Only, an association between IFN-γ and CD8 T cells was observed (**Fig. 8**) and has to be confirmed on larger study

- On clinical standpoint, **low T cell functionality at D1/2 in SS patients seems to be associated with severity/ mortality.** 3 patients died amongst 4 categorized with low IFNγ secretion (< 2IU/ml) at D1/2 (**Fig.9**). The other non survivor had an IFN at 2,77IU/mL. Interestingly, there is no significant correlation between SOFA score and IFN-γ (Fig.10)

Fig.5

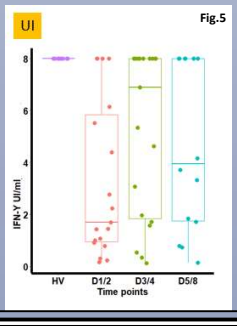


Fig. 6

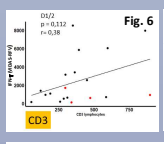


Fig. 7

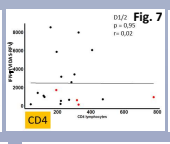


Fig. 8

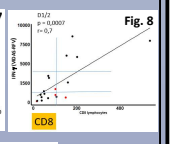


Fig. 9

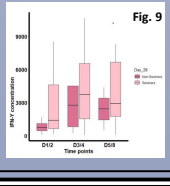
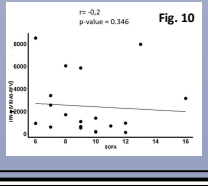


Fig. 10



Conclusion

VIDAS® STIMM™ T RUO is a new and powerful tool for monitoring antigen-independent T cell functionality in only 4 hours and in a simple, standardized, fully automated way. This solution was well integrated in a routine laboratory without any failure in 5 months and may democratize the assessment of cellular immunity in the future.

Further investigations are now needed to optimize the cut-offs and determine how the assay can be used to tailor treatment in patients with various immunosuppressive conditions: transplant, sepsis, cancer, immunotherapy…

33rd ECCMID EUROPEAN CONGRESS OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES
Copenhagen, Denmark
15–18 April 2023