The NTM-*i*Spot and its potential in the management of pulmonary NTM infections

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Preliminary results on the NTM-*i*Spot indicate that such test may have the potential to help guide the clinical evaluation of patients with NTM present in pulmonary samples but of unclear clinical relevance.

Background

Although commonly considered as clinically less relevant, infections by nontuberculous mycobacteria (NTM) represent a challenge for respiratory and infectious diseases physicians. Their incidence is increasing globally and determining their clinical relevance when found in pulmonary samples remains difficult. Considering the promising performance of a previous enzymatic NTM-specific EliSpot in pulmonary and extrapulmonary NTM-positive cases (pending publication), we decided to assess the use of this test further for patients with NTM isolates in pulmonary samples.

Methods

Using a recently developed NTM-specific 2-color fluorescence EliSpot (NTM-iSpot, GenID GmbH, Straßberg, Germany) we evaluated the immune response to NTM-specific antigens through T-cell production of IFN-γ and IL-2 (**Figure 1**). We tested 44 blood samples: 18 from patients with past or present NTM isolation in pulmonary samples (further classified as NTM-related disease, NTM colonization, and past NTM-related disease), 5 from patients with extrapulmonary NTM positive samples (3 lymphadenitis and 2 disseminated infections), 15 from patients with bronchiectasis with no record of NTM isolation, and 6 from healthy controls.
The results were interpreted using the Stimulation Index (SI=Number of spots after NTM-specific stimulation/Number of spots in the negative control) as follows. When there were 0-1 spot in the negative control the test was considered reactive if SI≥7; borderline if SI is >5 and <7; and not reactive if SI ≤5. When there were >1 spots in the negative control the test was considered: reactive if SI>3; borderline if SI is >2 to ≤3; and not reactive if SI ≤2. Responses in the mitogen below 50 spots and in the negative contol above 10 were considered invalid.

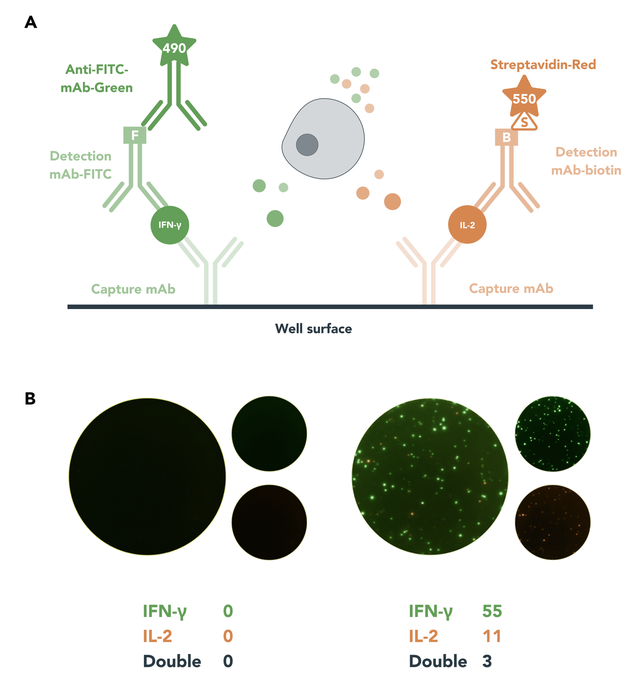

Figure 1. A. Schematic illustration of the two-color iSpot. B. Spot visualization. Representative pictures from a healthy control (left) and an patient with NTM pulmonary disease (right) tested with the NTM-*i*Spot. The healthy control shows no reactive T cells against NTM, while the NTM positive donor shows reactivity with functional T cells expressing both IFN-γ and IL-2. The small sized images show the IFN-γ and the IL-2 response separately (upper and lower images, respectively). The big size image shows the overlay of both cytokines.

Results

The extrapulmonary NTM-positive group had the highest rate of positive results followed by the pulmonary NTM-positive group (**Figure 2** and **Tables 1A** and **1B**). Within the latter group, the majority of positive results were found in the NTM-related disease cohort (55.5% and 30.0% for IFN-γ and IL-2 response, respectively). In the colonization group, one sample was reactive in terms of IL-2 response (SI value near the borderline) which was borderline in terms of IFN-γ. No reactive results were obtained in the groups of past NTM-related pulmonary disease, bronchiectasis without record of NTM isolation, and healthy controls.

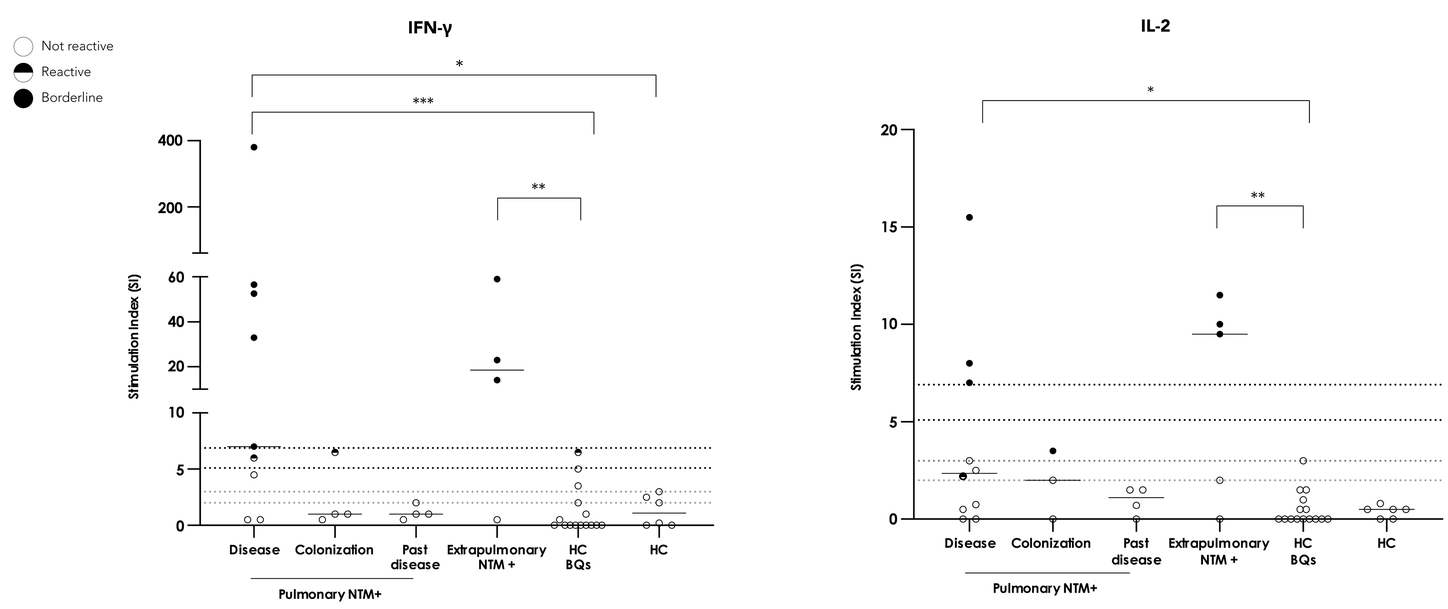

Figure 2. IFN-γ and IL-2 responses after NTM-specific stimulation. *= p<0.05, **= p<0.01, ***= p<0.001

The NTM species present in the NTM positive samples were varied (**Table 2**). Although the frequency of each specie in this sample is not high, preliminary results do not show association between the species and the NTM-*i*Spot result.

Table 2. NTM-*i*Spot results by NTM specie and patient group. Here, both reactive and borderline results considering IFN-γ and/or IL-2 response.

	NTM-iSpot (n=23) Reactive/Borderline IFN-γ &/or IL-2 (%)	
Pulmonary NTM+ (n=18)		
Disease (n=10)	6 (60.0)	
M. chimaera (n=4)		3 (75.0)
M. avium intracellulare (n=2)		2 (100)
M. kansasii (n=2)		0 (0.0)
M. abscessus (n=1)		0 (0.0)
M. xenopi (n=1)		1 (100)
Colonization (n=4)	1 (25.0)	
M. fortuitum (n=2)		1 (50.0)
M. palustre (n=1)		0 (0.0)
M. avium (n=1)		0 (0.0)
Past disease (n=4)	0 (0.0)	
M. chelonae[a] (n=2)		0 (0.0)
M. avium intracellulare (n=1)		0 (0.0)
M. abscessus (n=1)		0 (0.0)
Extrapulmonary NTM+ (n=5)[b]	3 (60.0)	
M. chimaera (n=2)		1 (50.0)
M. lentiflavum (n=2)		1 (50.0)
M. avium (n=1)		1 (100)

[a] One also positive for *M. malmoense*. [b]Three adenitis (2 by *M. lentiflavum* and 1 by *M. avium*) and two disseminated (by *M. chimaera*).

Table 1. NTM-*i*Spot results considering IFN-γ (A) and IL-2 (B) responses. Invalid results have been excluded.

A

	NTM-*i*Spot IFN-γ (n=42) Reactive (%)	Reactive/ Borderline (%)
Pulmonary NTM+ (n=17)	5 (29.4)	7 (41.2)
Disease (n=9)	5 (55.5)	6 (66.7)
Colonization (n=4)	0 (0.0)	1 (25.0)
Past disease (n=4)	0 (0.0)	0 (0.0)
Extrapulmonary NTM + (n=4)	3 (75.0)	3 (75.0)
HC BQs (n=15)	0 (0.0)	1 (6.7)
HC (n=6)	0 (0.0)	0 (0.0)

B

	NTM-*i*Spot IL-2 (n=43) Reactive (%)	Reactive/ Borderline (%)
Pulmonary NTM+ (n=17)	4 (23.5)	5 (29.4)
Disease (n=10)	3 (30.0)	4 (40.0)
Colonization (n=3)	1 (33.3)	1 (33.3)
Past disease (n=4)	0 (0.0)	0 (0.0)
Extrapulmonary NTM + (n=5)	3 (60.0)	3 (60.0)
HC BQs (n= 15)	0 (0.0)	0 (0.0)
HC (n=6)	0 (0.0)	0 (0.0)