Sistema Socio Sanitario
Regione Lombardia
ASST Ovest Milanese

QUANTIFICATION OF CD64: A PREDICTIVE BIOMARKER FOR PROGRESSION TO ACTIVE TUBERCULOSIS

CROI Seattle, Washington March 4-7, 2019

0716

Cristina Ceriani[1,2], Arianna Gatti[1,3], Massimo Villa[4], Maria Teresa Manco[1], Massimo De Paschale[1], Ilaria Caramma[4], Bruno Brando[3], Pierangelo Clerici[1]

[1] *Microbiology Unit, ASST Ovest Milanese, Legnano, Italy;* [2] *University of Milan, Milan, Italy;* [3] *Hematology Laboratory and Transfusion Center, ASST Ovest Milanese, Legnano, Italy;* [4] *Infectious Disease Unit, ASST Ovest Milanese, Legnano, Italy;*

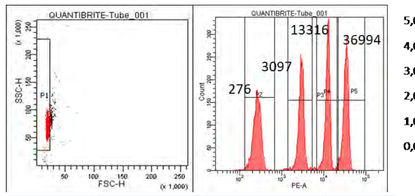
BACKGROUND

To reach the goal of end-Tuberculosis strategy, new biomarkers are needed to identify active tuberculosis (TB). Currently, only **QuantiFERON® TB assay (QTF)** is used in peripheral blood for screening tuberculosis infection. However, this method **cannot distinguish active from latent TB infection (LTBI).** Recent studies show a transcriptional increase of several genes, including **CD*64***, which code for a high affinity Fcγ receptor I **involved in inflammatory reactions**. In addition, Neutrophils (NE) and Monocytes (MO) exert bactericidal responses by producing inflammatory proteins caused by infection with *M. Tuberculosis* (MBT).

The purpose of this study was to **quantify CD*64* expression on the surface of NE and MO as a predictive biomarker of progression from LTBI to active TB** in QuantiFERON® (QTF) positive or indeterminate patients.

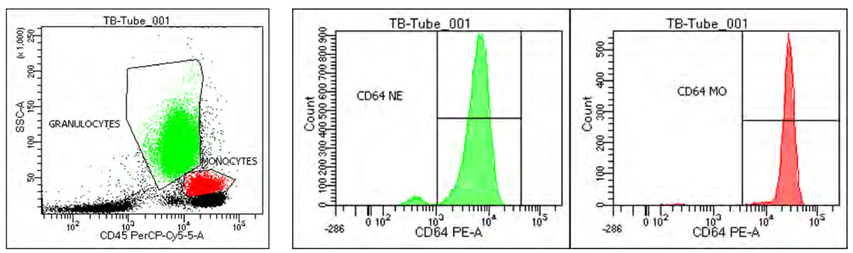
METHODS

47 patient were enrolled: 41 QTF positive and 6 QTF indeterminate. Non-systemic infections were documented. **Flow cytometric quantitative expression of CD*64*** was evaluated from peripheral blood samples with a combination of anti-CD64-PE (clone MD22) and anti-CD45-PerCP and expressed in ABC (Antibody Binding Capacity) units. NE normal range is <1000 ABC and MO normal range is 15000-20000 ABC. MTB cultures from respiratory specimens were also performed.



FL2 GEO MEAN	PE BEADS ABC	X Log PE BEADS	Y LOG PE FL2
276	474	2.676	2.441
3097	5359	3.729	3.491
13316	23843	4.377	4.124
36994	62336	4.795	4.568

Figure 1: Calibration of quantitative PE fluorescence was performed with four populations of beads loaded with known levels of PE (red histogram plot).



Specimen Name: TB

Population	CD64 PE-A Geo Mean
☒ CD64 NE	5,352
☒ CD64 MO	23,021

	FL2 GEO MEAN	ABC
NE	5352	9238
MO	23021	39737

Figure 2: NE (green population) and MO (red population) were gated by Side Scatter and CD45 expression. The histogram plots showed PE fluorescence intensity of CD64 on NE population (green histogram) and on MO population (red histogram), identified as geometric mean (NE Geo Mean: 5.352 and MO Geo Mean: 23.021). The calibration curve was used to correlate mean arbitrary cytometric intensity units and PE units, expressed as ABC.

RESULTS

Of the 41 positive QTF cases, 25 MTB cultures were positive and 16 were negative. The positive QTF with negative MTB cultures were considered LTBI.

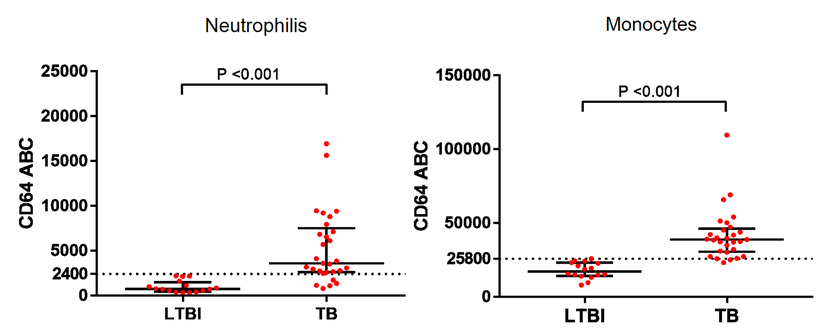


Figure 3: The median of NE CD64 ABC and MO CD64 ABC was significantly higher (p<0.001) in MTB positive cultures (NE 3593 ABC; MO 38757 ABC) than in MTB negative cultures (NE 724 ABC; MO 17151 ABC).

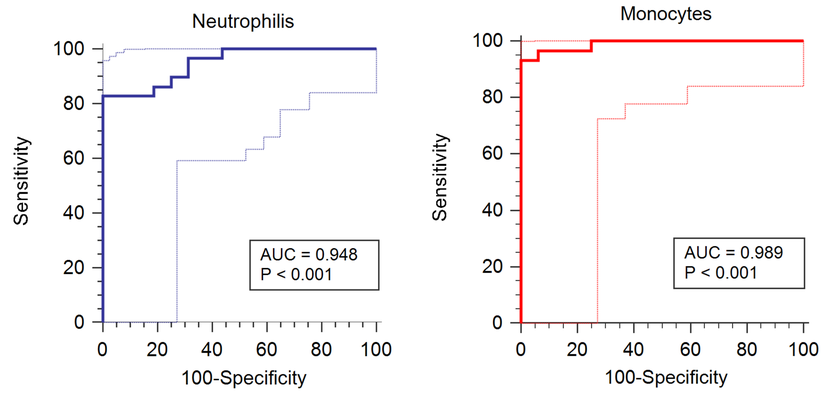


Figure 4: The NE CD64 and MO CD64 AUC-ROC values were 0.948 (95% CI 0.838-0.992) and 0.989 (0.901-1.000), respectively.
The difference between the two AUC-ROC are not statistically significant (pValue 0.1478).

Table 1: By establishing the NE CD64 value of >2400 ABC or the MO CD64 value of >25800, the sensitivity increased to 95.5% (82.2-99.9) with 100% specificity and 100% Positive Predictive Values (PPVs).

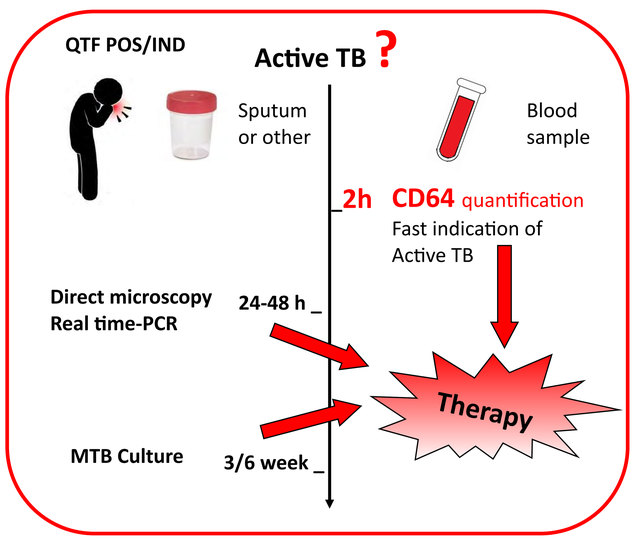
CD64	TP	FP	FN	TN	Sensibility % (IC 95%)	Specificity % (IC 95%)	PPV % (IC 95%)	NPV % (IC 95%)	Accuracy % (IC 95%)
NE >2400 ABC	24	0	5	16	82.76 (64.23-94.15)	100.00 (79.41-100.00)	100.00	76.19 (59.04-87.66)	88.89 (75.95-96.29)
MO >25800 ABC	27	0	2	16	93.10 (77.23-99.15)	100.00 (79.41-100.00)	100.00	88.89 (67.75-96.82)	95.56 (84.85-99.46)
NE >2400 ABC OR MO >25800 ABC	28	0	1	16	**96.55** (82.24-99.91)	**100.00** (79.41-100.00)	**100.00**	**94.12** (69.99-99.10)	**97.78** (88.23-99.94)
NE >2400 ABC AND MO >25800 ABC	23	0	6	16	79.31 (60.28-92.01)	100.00 (79.41-100.00)	100.00	72.73 (56.67-84.47)	86.67 (73.21-94.95)

Table 2: Of the 6 indeterminate QTF enrolled, the 4 with MTB positive cultures showed NE CD64 >2400 ABC or MO CD64 value >25800 ABC and the 2 with MTB negative cultures showed NE CD64 <1000 ABC or MO CD64 <23000 ABC.

ID	QTF	MTB Culture	MTB Real Time-PCR	Direct Microscopy	CD64 NE (ABC)	CD64 MO (ABC)
1	Ind	Neg	Neg	Neg	1139	18942
2	Ind	Neg	NA	Neg	592	21655
3	Ind	Pos	Pos	Pos	2885	29461
4	Ind	Pos	Pos	Pos	7889	48950
5	Ind	Pos	Pos	Pos	7412	60982
6	Ind	Pos	Pos	Pos	6278	49910
7	Ind	Pos	Pos	Pos	5399	44076

CONCLUSION

The quantification of NE and MO CD64 expression is a powerful diagnostic tool in discriminating between active TB and LTBI and may be used as predictive biomarker of active TB in patients with a positive QTF test. Providing a fast diagnostic solution may address the limitation of current tuberculosis diagnosis. Further studies with a larger patient cohort are needed to validate our preliminary data.



E-mail: cristina.ceriani87@gmail.com