A PREDICTIVE BIOMARKER FOR PROGRESSION TO ACTIVE TUBERCULOSIS

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BACKGROUND

To reach the goal of end-Tuberculosis strategy, new biomarkers are needed to identify active tuberculosis (TB). Currently, only Quantiferon® TB assay (QFT) 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 CD64, which code for a high affinity Fcy 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 (MTB).

The purpose of this study was to quantify CD64 expression on the surface of NE and MO as a predictive biomarker of progression from LTBI to active TB in Quantiferon® (QFT) positive or indeterminate patients.

METHODS

47 patient were enrolled: 41 QFT positive and 6 QFT indeterminate. Non-systemic infections were excluded. Flow cytometric quantitative expression of CD64 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. MTC cultures from respiratory specimens were also performed.

RESULTS

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

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 QFT 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.

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 QFT 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.

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

Figure 2: NE (green population) and MO (red population) were gated by Side Scatter and CD45 expression. The histogram plots show 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.

Figure 3: The median of NE CD64 ABC and MO CD64 ABC was significantly higher (p<0.001) in MTC positive cultures (NE 1393 ABC; MO 38757 ABC) than in MTC 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 (p-value 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 (PPV).

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

Table 3: Sensibility % (IC 95%), Specificity % (IC 95%), PPV % (IC 95%), NPV % (IC 95%), Accuracy % (IC 95%).

Table 4: Sensibility % (IC 95%), Specificity % (IC 95%), PPV % (IC 95%), NPV % (IC 95%), Accuracy % (IC 95%).