THE SIMOA ASSAY FOR DETECTION OF CLOSTRIDIUM DIFFICILE TOXINS HAS A GREATER SENSITIVITY THAN THE CYTOTOXICITY ASSAY.

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Background- Objective

Clostridium difficile is a major agent responsible for healthcare associated diarrheaea. The European guidelines recommend the use of a two-step algorithm for the diagnosis of Clostridium difficile infection (CDI) based on a sensitive screening method (GDH detection or NAAT) followed by a more specific test detecting toxins. Commercial EIA tests for detection of toxins display a suboptimal sensitivity and currently cannot be used as standalone test. An ultrasensitive assay detecting free toxins A and B has been recently developed by bioMérieux using the single molecule array technology (SIMOA). The objective of this study was to evaluate the analytical performances of SIMOA toxins A and B assay.

Materials and Methods

We compared the sensitivity and specificity of the assay using 100 frozen stools of patients previously diagnosed with CDI by toxigenic culture (see Barbut et al., EJC/CMID 2017). Among these patients, 67 had a positive cytotoxicity assay (CTA) on MRC-5 cell culture and 33 a negative CTA. We also tested stools of 38 patients negative for C. difficile by culture and 32 patients harboring a non-toxigenic strain of C. difficile.

SIMOA: SIMOA consists of toxin capture by specific anti-toxin A and B antibodies coated on paramagnetic beads (Quanterix Corporation), followed by detection with specific antibody conjugated to an enzyme β-galactosidase. Beads are then incubated with enzyme substrate and individually isolated in microwells of an array for digital imaging.

The threshold of positive result with SIMOA was set up at 22 and 18.8 pg/ml for toxins A and B detection, respectively.

Fecal calprotectin concentrations were determined using a quantitative immunoassay according to the manufacturers’ instructions (Quanmxue, Bühmann).

Fecal lactoferrin concentrations were determined with a quantitative ELISA according to the manufacturers’ instructions (IBD Scan, TechLab, Blacksburg, VA).

Statistical analysis: Toxins titers were arbitrarily classified into 3 groups and compared to different biological and clinical parameters. Continuous variables were compared by the Kruskall Wallis test whereas categorical variable were compared by the Fisher exact test.

Results

Among the 67 patients with a positive CTA assay, only 3 (4.5%) were negative for both toxins A and B by SIMOA (fig.1). The remaining stool samples were positive for both toxins (n=59) or for toxin B only (n=5). Among the 33 patients with CDI but negative for CTA, 9 (27.3%) were positive for both toxins by SIMOA, 5 (15.1%) were only positive for toxin A and 2 (6.1%) were only positive for toxin B. Among the 38 patients negative for C. difficile by culture, 2 (5.2%) were positive for toxin A only with a very low titer (43 and 40 pg/ml). The 32 patients harboring a non-toxigenic strain of CDI were all negative for both toxins.

Toxins A and B concentrations determined by SIMOA were significantly correlated (Pearson correlation=0.89, p<0.001) (fig.2). There was a statistical association between levels of toxins A or B and digestive inflammation markers as determined by levels of fecal lactoferrin or calprotectin. Severity was not correlated to levels of toxin production whereas there was a trend for a higher mortality rate in patients with a level of toxins >1000 pg/ml (Table 1).

Conclusions

This study confirmed that the sensitivity of the SIMOA assay is higher than the cytotoxicity assay and can detect fecal toxins in 48.5 % of samples found to be negative using the CTA which is currently the reference technique for detecting free toxins from stools. C. difficile toxin detection using SIMOA technology has the potential to improve and simplify the CDI diagnosis.