Defining a testing algorithm to improve the laboratory diagnosis of CDI

Summary

We have carried out an observational diagnostic study in four NHS laboratories using routinely submitted diarrhoeal faecal samples (n = 12 441) being examined for evidence of CDI. The very large sample size has enabled determination to high degree of accuracy of the sensitivity and specificity of selected commercial assays for the laboratory detection of *C. difficile*. Assays were chosen to represent the main *C. difficile* bacterial/toxin target options (two toxin enzyme immunoassays (EIAs), toxin gene (NAAT) and glutamate dehydrogenase (GDH) EIA). We have used these data to determine the accuracy of diagnostic algorithms for the laboratory diagnosis of CDI. In order to understand and optimise the use of algorithms for the diagnosis of CDI, we have determined the relative clinical values of the two reference tests for *C. difficile* by determining their relationships with patient outcomes (30-day mortality and morbidity-associated laboratory measurements).

• Our study is ~20 times larger, in terms of samples tested, than the CEP study, which at the time of publication in 2009 was the largest such *C. difficile* laboratory test evaluation.

• *C. difficile* toxin EIAs are not suitable as stand alone tests for CDI. The two commonly used toxin EIAs included in this study were not equivalent. The Toxin A/B II, (TechLab) was significantly more sensitive than the Premier Toxin A + B (Meridian) (83.2, 95% C.I 80.2-85.8 vs 67.0, 95% C.I 62.9-70.9). This has clear implications for product selection if a toxin EIA is continued to be used (e.g. as part of an algorithm) for the diagnosis of CDI.

• Based on the results of the training phase, we initially explored the accuracy of two algorithms, one optimising for sensitivity (GDH/NAAT) and the other optimising specificity (toxin EIA2/NAAT).

• As predicted, combining tests into two-stage algorithms increased the specificity (and so also the PPV) of results, but at the expense of a loss in sensitivity.

• Overall, the two-stage algorithm toxin EIA2/NAAT yielded the highest specificity when compared with either of the two reference tests (99.5% for both), although the GDH/NAAT algorithm was more sensitive. The PPV for the toxin based algorithm was therefore also higher (89.0-90.8%) than the GDH based algorithm (59.6-80.7%) compared with cell cytotoxin and cytotoxigenic culture references tests, respectively.

• There is a key dilemma whether to use a testing algorithm that is optimised for sensitivity, resulting in lowered specificity and PPV, or specificity, which compromises the sensitivity and NPV.

• Outcomes analyses show that cytotoxin test positive results correlate significantly with mortality (on multivariate analysis). Thus, (cyto)toxin detection is a better indicator of disease than the presence of toxigenic strains. The caveat is that some patients with

genuine CDI will be missed if diagnosis is based ultimately on toxin detection. Culture of toxigenic *C. difficile* in the absence of toxin (i.e. cytotoxigenic culture positive, cytotoxin negative) was not associated with any significant clinical outcome worse than that of *C. difficile* negative samples. However, samples with *C. difficile* but no demonstrable toxin can indicate potential *C. difficile* excretors, and this may aid infection prevention and control measures.

• One way to help address the issue of missing true CDI diagnoses, when using an algorithm that is optimised for specificity, is to use a different approach for the reporting and interpretation of samples that are positive by the first but not the second test. A sample that is positive by the first (screening) test e.g. GDH or NAAT, but is negative by a toxin test could be used to indicate a higher index of suspicion that CDI may be present. The overall performance of a GDH/EIA2 algorithm (PPV 91.4%, NPV 98.9%) is actually very similar to that of the specificity optimised algorithm, but has the advantage of being able to indicate when CDI is present, when *C. difficile* could be present and when neither of these scenarios exists.

• As an alternative to optimising specificity, combining non-toxin targeted tests (GDH/NAAT) results in a high sensitivity algorithm, although this is marginally lower than using the standalone tests e.g. 95.2% for GDH/NAAT vs 96.4% for GDH alone when compared with the cell cytotoxin test. Combining these tests improves the specificity (and PPV) but these are still poor overall 95.9% (59.6%) vs 92.2% (43.8%). Such algorithm test results are useful for ruling out infection, but are poor at determining if it is truly present. This presents potential diagnostic (reporting) and treatment dilemmas.

• The ultimate choice of test algorithm from our study depends on the clinical context and particularly the balance between acting on a positive result for patients with presumed CDI and the use of clinical judgement.

• Accurate diagnosis of CDI is important for patient management, infection prevention and control, and for the attainment of reliable surveillance data. Recommending one algorithm approach will require clear messages to be made available to laboratories, clinicians and epidemiologists, particularly concerning the PPV and NPVs of test results.

• No test or combination of tests is infallible and the clinical condition of the patient should always be taken into consideration when making management choices.

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November 2011