Is there any value in measuring faecal calprotectin in \textit{Clostridium difficile} positive faecal samples?

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Markers of intestinal inflammation have been proposed for inclusion in \textit{Clostridium difficile} diagnostic algorithms. Faecal calprotectin (f-Cp), a sensitive marker of intestinal inflammation, was evaluated for utility in \textit{C. difficile} diagnosis in the hospital setting. One hundred and twenty \textit{C. difficile} positive and 99 \textit{C. difficile} negative faecal samples of hospital-acquired diarrhoea were analysed for f-Cp using a quantitative ELISA. \textit{C. difficile} positivity was confirmed using ELISAs for either toxins (n=45) or glutamate dehydrogenase (GDH) with toxin gene confirmation (n=75). Non-parametric ANOVA (Kruskal–Wallis) was used for data analysis. \textit{C. difficile} positive samples had higher (P<0.05) median (interquartile range) f-Cp levels; 336 \textmu g g\textsuperscript{-1} (208–536) for toxin and 249 \textmu g g\textsuperscript{-1} (155–498) for GDH and toxin gene positive compared with 106 \textmu g g\textsuperscript{-1} (46–176) for \textit{C. difficile} and culture-negative faecal samples. Five \textit{C. difficile} positive samples were f-Cp negative (<50 \textmu g g\textsuperscript{-1}). A f-Cp concentration >50 \textmu g g\textsuperscript{-1} was 96 \% sensitive and 28 \% specific for \textit{C. difficile}, with area under the ROC curve of 0.82. There is no role for f-CP alone in predicting \textit{C. difficile} infection in hospital-acquired diarrhoea due to its low specificity.

\textbf{INTRODUCTION}

\textit{Clostridium difficile} is the commonest cause of hospital-acquired diarrhoea (Barbut \textit{et al.}, 1996) and is associated with significant morbidity and mortality. The toxins produced by the bacterium cause intestinal inflammation and cell death. Approximately 20 \% of hospital inpatients over the age of 65 years are carriers of the bacterium (Healthcare Commission, 2006), with certain antibiotic-resistant strains now becoming epidemic (He \textit{et al.}, 2013). Prompt diagnosis is essential to guide rapid and appropriate treatment for the patient and allow steps to be taken to prevent spread. Due to lack of specificity and sensitivity in the available tests, differentiating asymptomatic colonization from pathogenic infection, particularly in those with diarrhoea, may be difficult.

Reference methods for \textit{C. difficile} infection are cell culture toxicity assay and toxigenic culture taking between 24 and 48 h where both presence and toxigenicity of the strain are determined (Crobach \textit{et al.}, 2009). Alternatively immunoassays can be used to detect either \textit{C. difficile} products, e.g. glutamate dehydrogenase (GDH), or toxins, having shorter turn around times than culture methods (Crobach \textit{et al.}, 2009). Toxin detecting assays have good specificity (majority >95 \%); however, sensitivity varies from 40 to 100 \%, introducing the chance of false negatives (Burnham & Carroll, 2013). Real-time PCR (RT-PCR) and other nucleic acid amplification tests can be used to detect toxin-producing genes (Crobach \textit{et al.}, 2009). Tests may be used in combination (Crobach \textit{et al.}, 2009; Wilcox, 2012) and a two-step diagnostic algorithm has been recommended by the Department of Health (2012) to improve sensitivity and specificity of the commonly used toxin tests by introducing a screening stage. Detection of \textit{C. difficile} in the absence, compared with the presence, of toxin is associated with reduced mortality and symptom duration (Baker \textit{et al.}, 2013). Pathogenic strains of \textit{C. difficile}, however, may still be commensals and not producing toxin. To address this issue, the use of non-specific markers of intestinal inflammation, such as lactoferrin, has been suggested to indicate both the expression and effect of \textit{C. difficile} toxins (LaSala \textit{et al.}, 2013).

Calprotectin, a calcium- and zinc-binding protein found in neutrophils, is present at high levels in the stools of those with intestinal inflammation (Poullis \textit{et al.}, 2002). Faecal calprotectin (f-Cp) is the most sensitive and specific routinely available marker for intestinal inflammation (Lamb \textit{et al.}, 2011). The aim of this study was to evaluate the use of f-Cp in the diagnosis of \textit{C. difficile}. More specifically we investigated whether f-Cp may be used, in practice, to predict \textit{C. difficile} toxin or GDH antigen test

\textbf{Abbreviations:} f-Cp, faecal calprotectin; GDH, glutamate dehydrogenase; RT-PCR reverse transcriptase polymerase chain reaction.
positivity and whether f-Cp has a role within a diagnostic algorithm.

**METHODS**

A series of *C. difficile* positive (n=120) and negative (n=99) Bristol Stool Chart types 6 and 7 faecal samples collected from inpatients, aged 21 or over, in a 700-bed teaching hospital, were studied after being made anonymous. The departmental *C. difficile* testing methodology was changed during the evaluation, resulting in two study phases. During phase 1 (n=75), *C. difficile* positivity was detected by a bioMérieux Clinical Diagnostics Vidas *C. difficile* toxin ELISA. During phase 2 (n=45) a two-step algorithm was used whereby samples were first tested for the GDH antigen using a TECHLAB C. diff Chek ELISA. If positive, samples were then tested for toxigenicity using a Cepheid Xpert reverse transcriptase PCR (RT-PCR) *C. difficile* toxin gene assay. Negative samples were all tested by the phase 2 protocol and therefore were negative for *C. difficile* GDH antigen. All samples were culture negative for parasites, *Escherichia coli* O157:H7, *Shigella*, *Salmonella*, *Verosinia* and *Campylobacter* spp. Faecal calprotectin was analysed using the quantitative Immunodiagnostik PhiCal ELISA; inter-batch coefficients of variation were 9.5 %, 7.0 % and 8.9 % at f-Cp levels of 53, 80 and 354 mg g⁻¹ respectively (unpublished data; Whitehead et al., 2013). A level of >50 mg g⁻¹ was considered to be positive (Lamb et al., 2011).

Data were non-parametric, as assessed by the Kolmogorov–Smirnov test. The Kruskal–Wallis test was, therefore, used to assess significance of variation, P-values <0.05 being considered statistically significant. Data processing and statistical analysis were performed using GraphPad Prism version 4.00 (GraphPad Software) and Analyse-it version 2.22 (Analyse-it Software). Results are expressed as medians with inter-quartile ranges.

**RESULTS**

F-Cp concentrations were higher (P<0.05) in the 75 toxin ELISA positive samples (336; 208–536 mg g⁻¹) than in the 45 GDH ELISA/toxin gene RT-PCR positive samples (249; 155–498 mg g⁻¹) and both were higher (P<0.05) than in the 99 *C. difficile* negative samples (106; 46–176 mg g⁻¹). F-Cp concentrations were elevated (>50 mg g⁻¹) in 95 % and 98 % of *C. difficile* positive samples identified using the toxin ELISA and GDH ELISA/toxin gene methods respectively (Fig. 1). Five *C. difficile* positive samples had f-Cp concentrations <50 mg g⁻¹; of these, four were from the toxin ELISA method group (Fig. 1). F-Cp was positive in 74 % of the *C. difficile* negative samples (Fig. 1). At a value of 50 mg g⁻¹, f-Cp had sensitivities of 98 % and 95 % for A/B toxin ELISA and GDH ELISA/toxin gene PCR positivity, respectively, but the specificity at this cut-off was only 26 % in both groups.

Receiver operator characteristic (ROC) curves for f-Cp as a diagnostic indicator for *C. difficile* positivity utilizing either A/B toxin ELISA or GDH ELISA/toxin gene PCR are shown in Fig. 2. In summary, ROC analysis gave an area under the curve of 0.84 and 0.80 for *C. difficile* positivity utilizing A/B toxin ELISA and GDH ELISA/toxin gene PCR, respectively. The optimum f-Cp value was 176 mg g⁻¹ with a sensitivity of 81 % and specificity of 77 % for A/B toxin ELISA positivity and 169 mg g⁻¹ with a sensitivity of 73 % and specificity of 77 % for GDH ELISA/toxin gene PCR positivity.

**DISCUSSION**

Our data, based on widely used diagnostic strategies, do not support a role for f-Cp in excluding *C. difficile* infection as a cause of hospital-acquired diarrhoea. Although sensitive
for *C. difficile* infection, f-Cp lacked specificity, since most (74%) hospital-acquired diarrhoea, even in the absence of bacterial and parasitic infection, was associated with raised f-Cp concentrations. Although not directly comparable, our results appear to differ from previous reports. Shastri *et al.* (2008) reported that f-Cp was 83% sensitive and 87% specific for acute bacterial gastroenteritis and performed better than faecal lactoferrin (78% sensitivity and 54% specificity). Sýkora *et al.* (2010) reported that f-Cp had a sensitivity of 93% and specificity of 88% in predicting bacterial infection in young children. The differences may relate to the different study populations; our study was limited to hospitalized patients, whereas the other two studies included patients largely drawn from the community.

The raised f-Cp concentrations in most subjects in our study also indicate that patients admitted with diarrhoea, or who acquire diarrhoea in hospital, tend to have intestinal inflammation irrespective of its aetiology. The higher f-Cp concentrations in *C. difficile* positive than in *C. difficile* negative diarrhoea indicate greater intestinal inflammation, which may underpin the significant morbidity and mortality associated with *C. difficile* infection.

The role of f-Cp as part of a diagnostic algorithm to differentiate between colonization by pathogenic *C. difficile* and infection was also investigated. Only five *C. difficile* positive patients had normal f-Cp concentrations (<30 μg g⁻¹), of which four were in the toxin ELISA method group. Toxin ELISA methods are associated with the risk of false negatives, rather than false positives, given their low sensitivity and high specificity (Burnham & Carroll, 2013). We therefore speculate that these four cases may represent mild disease, as previously suggested (Wren *et al.*, 2009), since increased f-Cp in patients with diarrhoea is associated with greater severity of gut inflammation (Chen *et al.*, 2012; Nielsen *et al.*, 2013). Similarly the one GDH ELISA/toxin gene positive but f-Cp negative case could represent mild disease but may also be due to a commensal non-toxin secreting pathogenic strain of *C. difficile*. The data, however, do not support a role for f-Cp in *C. difficile* diagnostic algorithms as the presence of *C. difficile*, particularly with the new two-step GDH ELISA/toxin gene RT-PCR diagnostic strategy, is practically always associated with intestinal inflammation. Subsequent to this study, local practice, in order to discriminate commensal pathogenic strains from toxin-producing infections, has continued to use the phase 2 algorithm (GDH ELISA/toxin gene PCR) to identify positive cases to treat but to confirm toxin production with toxin ELISA in a batched weekly assay for national reporting purposes.

In conclusion, this study does not support a role for f-Cp alone in predicting *C. difficile* diagnostic test positivity in hospital-acquired diarrhoea due to its low specificity.

**ACKNOWLEDGEMENTS**

We thank the staff of New Cross Hospital Microbiology department for their help in collecting samples.

**REFERENCES**


