Comparison of serum and whole-blood specimens for the detection of *Candida* DNA in critically ill, non-neutropenic patients

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In contrast to the multitude of studies on fungal PCR assay methods, little work has been reported evaluating *Candida* PCR performance when using whole blood compared with serum in candidaemic patients. Here, a comparison of the performance of whole-blood and serum specimens using a set of real-time PCR *Candida* species assays is described. Specimens were collected prospectively from non-neutropenic adults who were recruited to a diagnostic clinical trial, the primary purpose of which was to verify the performance of the assays using serum; in all, 104 participants also had whole-blood specimens submitted for analysis in addition to the serum specimen. Of these participants, 10 had laboratory-confirmed candidaemia and 94 were categorized as being 'unlikely' to have invasive *Candida* infection. PCR results from the whole-blood specimens are presented here and compared with the results from serum specimens in this subgroup among whom both specimen types were obtained contemporaneously. All participants with candidaemia were PCR-positive from serum samples; however, only seven were PCR-positive from whole blood. All specimens from patients in the 'unlikely' category were PCR-negative in both types of specimen. Moreover, DNA extraction from serum required 1 h; extraction from whole blood required approximately 3 h. These data tentatively suggest that, overall, serum is an appropriate specimen for *Candida* PCR for detection of candidaemia in non-neutropenic adults.

Received 3 April 2008

Accepted 4 June 2008

INTRODUCTION

Despite advances during the last decade, invasive fungal infections are still associated with a high morbidity and mortality in immunocompromised and critically ill patients (Hsiao *et al.*, 2006; Pugliese *et al.*, 2007). Considerable progress has been made in non-culture diagnostics, particularly in genomic amplification methods. However, evaluation of new non-culture methods has focused mainly on patients with haematological malignancies or bone marrow transplantation (Karthaus & Cornely, 2004; Ribeiro *et al.*, 2006; White *et al.*, 2006) and few studies have evaluated fungal PCR performance in non-neutropenic patients; moreover, no direct comparison has been carried out between different blood fractions for PCR testing for *Candida* species detection in clinical trials.

One of the critical factors for a successful *Candida* PCR assay in patients with candidaemia is extraction of sufficient nucleic acid from the clinical specimen. Several fungal PCR methods have been developed for use on whole-blood or serum samples (Chryssanthou *et al.*, 1994;

van Burik *et al.*, 1998; Wahyuningsih *et al.*, 2000; Schabereiter-Gurtner *et al.*, 2007). However, only one study has compared the sensitivity of the two; this was for detection of *Aspergillus* DNA in neutropenic patients (Löffler *et al.*, 2000). There are no available data comparing serum and blood for detection of *Candida* DNA in non-neutropenic patients.

Here, we describe a comparison between serum and whole blood for detection of *Candida* species DNA in non-neutropenic, critically ill adults.

METHODS

A substantial proportion of the data presented here was obtained in the course of a prospective diagnostic clinical trial in a single critical care unit (McMullan *et al.*, 2008). The main purpose of that trial was to verify the clinical performance of a set of three real-time PCR assays for detection of *Candida* DNA from serum specimens; 157 participants were recruited from whom 527 serum specimens were obtained. Of these, 104 participants had a whole-blood specimen submitted for analysis contemporaneously with the serum specimen.

The data relating to the whole-blood specimens were not included in the description of the original trial, as it was not principally intended to evaluate this specimen type. The present report compares PCR performance in whole-blood specimens from this subset of 104 participants with that in the paired serum specimens already reported as part of the original trial.

In all, 10 pairs of specimens were submitted from participants with laboratory-proven candidaemia, whilst 94 pairs were from participants categorized as being 'unlikely' to have invasive *Candida* infection. Specimens were kept frozen at -80°C until extracted. DNA was extracted from 3 ml EDTA-treated whole blood according to the method published by Löffler *et al.* (1997) as this method has been extensively used and described in the literature for extraction of fungal DNA from EDTA-treated blood. An extraction control was processed in parallel with each batch of samples. Extractions from serum samples were performed as described by McMullan *et al.* (2008) using a QIAamp DNA Mini kit (Qiagen) and carrier RNA. Nucleic acids were eluted in AE buffer (provided in the kit) and stored at -20°C until used as template for PCR.

Our previously published set of nested Taqman-based real-time PCR assays (McMullan *et al.* 2008) allows the detection of six medically important *Candida* species. *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis* and *Candida dubliniensis* were detected in the first assay, whilst *Candida glabrata* and *Candida krusei* were detected in the second and third assays, respectively. The assays were applied to DNA extracted from both specimen types. A positive result for either serum or blood was categorized as PCR-positive. For exclusion of PCR inhibition, all PCR-negative specimens from candidaemic participants were tested again after spiking the sample DNA with an internal amplification control consisting of a cloned *Escherichia coli* random PCR product constructed according to the method described by Bohlander *et al.* (1992). The spiked extract was amplified using the single primer 5'-GTTTCCCAGTCACGATC-3' and detected by gel electrophoresis.

RESULTS AND DISCUSSION

The data were evaluated with a view to informing the choice of blood fraction employed for PCR-based detection of *Candida* DNA in blood from non-neutropenic patients.

Both reliability and ease of use were evaluated in this context.

Paired specimens from 104 participants were analysed independently using *Candida* species nested real-time PCR assays. Extraction of DNA from serum required approximately 1 h, whilst that from whole blood required approximately 3 h; the difference is largely explained by the time required to achieve lysis of erythrocytes, leukocytes and the fungal cell wall. All specimens from the 'unlikely' group were PCR-negative. As shown in Table 1, *Candida* DNA was detected in serum samples from all ten of the patients with proven candidaemia; however, only seven of the ten matched whole-blood samples were PCR-positive. Of the three whole-blood specimens that falsely tested negative, two had been taken on the same day as the positive serum culture and one had been taken on the following day; it appears that these cannot, therefore, readily be explained by asynchronous sampling. Seven of the ten participants with proven candidaemia had *C. albicans* infection and three had *C. glabrata* infection; of the three with whole-blood specimens that tested negative, two had *C. albicans* infection and one had *C. glabrata* infection. Using the method described, PCR inhibition was not found in these three specimens.

It has been postulated by Bougnoux *et al.* (1999) and Kasai *et al.* (2006) that cell-free fungal DNA is present in the bloodstream of patients with invasive candidiasis and that this may readily be extracted from serum; however, it is noteworthy that this is based on an experimental rabbit model. Notwithstanding this, our previously reported data provide clinical support for the applicability of serum in this context to humans (McMullan *et al.*, 2008).

To our knowledge, the only other report that has formally compared different blood fractions for detection of fungal DNA in clinical specimens was published by Löffler *et al.*

Table 1. PCR results for patients with proven candidiasis

Patient no.	Age/sex	Underlying diagnosis category	Blood culture results	PCR results		Risk factors	
				Serum	Blood	Antibiotic*	CVC†
1	38/F	Respiratory	<i>C. albicans</i>	+	—	Present	Present
2	74/F	Cardiovascular	<i>C. glabrata</i>	+	+	Present	Present
3	76/M	Not classified	<i>C. albicans</i>	+	+	Present	Present
4	76/M	Cardiovascular	<i>C. albicans</i>	+	+	Present	Present
5	39/F	Dermatological‡	<i>C. albicans</i>	+	+	Present	Present
6	25/M	Trauma	<i>C. albicans</i>	+	—	Present	Present
7	71/F	Respiratory	<i>C. glabrata</i>	+	+	Present	Absent
8	75/M	Musculoskeletal	<i>C. glabrata</i>	+	—	Present	Absent
9	19/M	Dermatological‡	<i>C. albicans</i>	+	+	Present	Present
10	82/F	Cardiovascular	<i>C. albicans</i>	+	+	Present	Absent

*Antibiotic therapy in the last 30 days.

†Central venous catheter.

‡Burn.

(2000); plasma PCR was found to be less sensitive than PCR performed on whole blood for detection of *Aspergillus* DNA in neutropenic patients. However, these findings cannot readily be generalized to candidaemic patients, and the data presented here suggest that this pattern may not hold true for candidaemia. Furthermore, studies of the kinetics of DNA release by *Candida* species demonstrate that cell-free fungal DNA is released into the bloodstream of hosts with disseminated candidiasis, and it seems that phagocytic cells play an active role in increasing this release over time (Bougnoux *et al.*, 1999; Kasai *et al.*, 2006). Consequently, the apparently better performance of serum samples in this study could perhaps be related to the fact that the patients were non-neutropenic. It may be that serum is a more reliable fraction in non-neutropenic subjects, whereas whole blood may be preferable in neutropenic individuals. Moreover, it may be that one particular specimen type yields a positive result earlier in the disease course; however, this could not be evaluated in our small dataset. Further work aimed at addressing these issues could add substantially to the optimization of PCR assays in different patient populations – a trial to compare formally the two types of clinical specimens from candidaemic patients with and without neutropenia, preferably across a longer time period of the illness, could meet this need.

A notable limitation of this work was that, as it was conducted in a single centre over a short period of time, the number of specimens from candidaemic patients was limited by the incidence of candidaemia in our institution during the study (Metwally *et al.*, 2007). Furthermore, the comparison being reported here was not a principal objective of the diagnostic trial from which the data were obtained. Moreover, one cannot be assured that similar results would have been obtained if different extraction methods had been employed; we have previously reported marked variability in the performance of different methods for extraction of *Candida* DNA from whole blood (Metwally *et al.*, 2008). Nonetheless, these results provide intriguing pilot data in support of serum specimens with respect to both performance and time to achieve DNA extraction in candidaemic patients.

We conclude that testing serum specimens for the detection of *Candida* DNA in non-neutropenic candidaemic adults is appropriate; however, we are unable to generalize this finding to other groups or to draw any inference of superiority. Further investigation is required to reach firm conclusions about the optimal choice of specimen with respect to cost, performance and laboratory workflow impact.

ACKNOWLEDGEMENTS

This work was supported by the Northern Ireland Health and Personal Social Services Research and Development Office as part of commissioned research under the antimicrobial resistance action plan.

REFERENCES

- Bohlander, S. K., Espinosa, R., III, Le Beau, M. M., Rowley, J. D. & Diaz, M. O. (1992). A method for the rapid sequence-independent amplification of microdissected chromosomal material. *Genomics* **13**, 1322–1324.
- Bougnoux, M., Dupont, C., Mateo, J., Saulnier, P., Faivre, V., Payen, D. & Nicolas-Chanoine, M. (1999). Serum is more suitable than whole blood for diagnosis of systemic candidiasis by nested PCR. *J Clin Microbiol* **37**, 925–930.
- Chrysanthou, E., Andersson, B., Petrini, B., Löfdahl, S. & Tollemar, J. (1994). Detection of *Candida albicans* DNA in serum by polymerase chain reaction. *Scand J Infect Dis* **26**, 479–485.
- Hsiao, H. H., Tsai, H. J., Liu, Y. C., Tseng, Y. T., Lu, P. L., Yang, W. C., Liu, T. C. & Lin, S. F. (2006). Invasive fungal infections in patients with acute leukemia. *Kaohsiung J Med Sci* **22**, 217–222.
- Karthus, M. & Cornely, O. A. (2004). Recent developments in the management of invasive fungal infections in patients with hematological malignancies. *Ann Hematol* **84**, 207–216.
- Kasai, M., Francesconi, A., Petraitienė, R., Petraitis, V., Kelaheer, A. M., Kim, H. S., Meletiadis, J., Sein, T., Bacher, J. & other authors (2006). Use of quantitative real-time PCR to study the kinetics of extracellular DNA released from *Candida albicans*, with implications for diagnosis of invasive candidiasis. *J Clin Microbiol* **44**, 143–150.
- Löffler, J., Hebart, H., Brauchle, U., Schumacher, U. & Einsele, H. (2000). Comparison between plasma and whole blood specimens for detection of *Aspergillus* DNA by PCR. *J Clin Microbiol* **38**, 3830–3833.
- Löffler, J., Hebart, H., Schumacher, U., Reitze, H. & Einsele, H. (1997). Comparison of different methods for extraction of DNA of fungal pathogens from cultures and blood. *J Clin Microbiol* **35**, 3311–3312.
- McMullan, R., Metwally, L., Coyle, P. V., Hedderwick, S., McCloskey, B., O'Neill, H. J., Patterson, C. C., Thompson, G., Webb, C. H. & other authors (2008). A prospective clinical trial of a real-time polymerase chain reaction assay for the diagnosis of candidemia in nonneutropenic, critically ill adults. *Clin Infect Dis* **46**, 890–896.
- Metwally, L., Walker, M. J., Coyle, P. V., Hay, R. J., Hedderwick, S., McCloskey, B. V., O'Neill, H. J., Webb, C. H. & McMullan, R. (2007). Trends in candidemia and antifungal susceptibility in a university hospital in Northern Ireland 2001–2006. *J Infect* **55**, 174–178.
- Metwally, L., Fairley, D. J., Coyle, P. V., Hay, R. J., Hedderwick, S., McCloskey, B., O'Neill, H. J., Webb, C. H., Elbaz, W. & McMullan, R. (2008). Improving molecular detection of *Candida* DNA in whole blood: comparison of seven fungal DNA extraction protocols using real-time PCR. *J Med Microbiol* **57**, 296–303.
- Pugliese, F., Ruberto, F., Cappannoli, A., Perrella, S. M., Bruno, K., Martelli, S., Marcellino, V., D'Alio, A., Diso, D. & other authors (2007). Incidence of fungal infections in a solid organ recipients dedicated intensive care unit. *Transplant Proc* **39**, 2005–2007.
- Ribeiro, P., Costa, F., Monteiro, A., Caldas, J., Silva, M., Ferreira, G., Veiga, J., Sousa, M. O., Viegas, M. P. & other authors (2006). Polymerase chain reaction screening for fungemia and/or invasive fungal infections in patients with hematologic malignancies. *Support Care Cancer* **14**, 469–474.
- Schabereiter-Gurtner, C., Selitsch, B., Rotter, M. L., Hirschl, A. M. & Willinger, B. (2007). Development of novel real-time PCR assays for detection and differentiation of eleven medically important *Aspergillus* and *Candida* species in clinical specimens. *J Clin Microbiol* **45**, 906–914.
- van Burik, J. A., Myerson, D., Schreckhise, R. W. & Bowden, R. A. (1998). Panfungal PCR assay for detection of fungal infection in human blood specimens. *J Clin Microbiol* **36**, 1169–1175.

Wahyuningsih, R., Freisleben, H. J., Sonntag, H. G. & Schnitzler, P. (2000). Simple and rapid detection of *Candida albicans* DNA in serum by PCR for diagnosis of invasive candidiasis. *J Clin Microbiol* **38**, 3016–3021.

White, P. L., Linton, C. J., Perry, M. D., Johnson, E. M. & Barnes, R. A. (2006). The evolution and evaluation of a whole blood polymerase chain reaction assay for the detection of invasive aspergillosis in hematology patients in a routine clinical setting. *Clin Infect Dis* **42**, 479–486.