Pathogen-specific DNA enrichment does not increase sensitivity of PCR for diagnosis of invasive aspergillosis in neutropenic patients.

Springer J, Loeffler J, Heinz W, Schlossnagel H, Lehmann M, Morton O, Rogers TR, Schmitt C, Frosch M, Einsele H, Kurzai O (2011) Pathogen-specific DNA enrichment does not increase sensitivity of PCR for diagnosis of invasive aspergillosis in neutropenic patients. *J Clin Microbiol* 49(4), 1267-1273.

Abstract

PCR assays designed for the diagnosis of invasive aspergillosis (IA) in high-risk patients have to detect minute amounts of target DNA to reach sufficient analytical sensitivity to be of clinical use. This prospective study assessed the use of a novel strategy for selective pathogen DNA enrichment for enhancing the performance of diagnostic PCR in a direct comparison with a highly sensitive in-house quantitative PCR (qPCR) assay and the galactomannan enzyme-linked immunosorbent assay (ELISA). Surprisingly, and in contrast to experience with other patient groups, the novel protocol for selective pathogen DNA enrichment did not enhance but instead significantly impaired sensitivity. This could be explained by the small amounts of host DNA in the specimens, which were derived mostly from severely neutropenic patients. In the qPCR assay, positive samples required an average of 43.5 amplification cycles (range, 39.2 to 50) for detection in the in-house PCR. Repetitive testing of selected samples showed test positivity to be variable,

most likely due to the small amounts of target DNA. Despite this, the in-house protocol proved helpful in the diagnosis of IA, detecting 2 out of 3 patients with probable IA and 10 out of 19 patients with possible IA. Our results underline the necessity for diagnostic PCR protocols that help diagnose IA to be highly sensitive and show that selective pathogen DNA enrichment using affinity purification may not be useful in severely neutropenic patients.

Involved units

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Details

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