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Baseline cytokine profiling identifies novel risk factors for invasive fungal disease among haematology patients undergoing intensive chemotherapy or haematopoietic stem cell transplantation

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Summary *Background:* Invasive fungal disease (IFD) is a disease of immunocompromised hosts. Cytokines are important mediators of innate and adaptive immune system. The aim of this study was to identify cytokine profiles that correlate with increased risk of IFD.
Methods: We prospectively enrolled 172 adult haematology patients undergoing intensive chemotherapy, immunosuppressive therapy, and haematopoietic stem cell transplantation. Pro-inflammatory cytokine profiling using 30-plex Luminex assay was performed at baseline and during treatment. Nine single nucleotide polymorphisms (TLR1, TLR2, TLR3, TLR4.1, TLR4.2, TLR6, CLEC7A, CARD9, and INFG) were investigated among transplant recipients and donors.
Findings: The incidence of IFD in this cohort was 16.9% (29/172). Median baseline serum concentrations of IL-15, IL-2R, CCL2, and MIP-1α were significantly higher whilst IL-4 was lower in patients with proven/probable IFD compared to those with no evidence of IFD. Baseline high

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IL-2R and CCL2 were associated with increased risk of IFD in the multivariate analysis (adjusted hazard ratio 2.3 [95% CI 1.1—5.1; P = 0.037], and hazard ratio 2.7 [95% CI 1.2—6.1; P = 0.016], respectively). However, these differences were not significant in follow up measurements. Similarly, no significant independent prognostic value was associated with baseline cytokine profile.

Interpretation: High baseline IL-2R and CCL2 concentrations were independent indicators of the risk of developing IFD and could be used to identify patients for enhanced prophylaxis and early antifungal therapy.

Introduction

Invasive fungal disease (IFD) remains an important cause of mortality and morbidity among haematology patients undergoing chemotherapy and haematopoietic stem cell transplantation (HSCT).[1—3] This is largely due to a defective immune system caused by the underlying primary haematological malignancies and their often complex treatments.[4] IFD caused by moulds such as invasive aspergillosis (IA) are notoriously difficult to diagnose in clinical practice and indeed no validated standardised criteria exist for this purpose.

The ubiquitous nature of moulds makes human contact almost universal. Inhalation of asexual spores (conidia) brings them into contact with the respiratory macrophages and monocytes, which are the first line of defence against IFD.[5] These effector cells of the innate immune system respond to the presence of the conidia, in concert with neutrophils, by a variety of complex mechanisms such as phagocytosis and intracellularly destroying conidia, and aggregation around the conidia to prevent their germination.[5—7] The innate immune system relies on pattern recognition receptors (PRR) on the surface of immune cells, which recognise pathogen-associated molecular patterns (PAMP) on the surface of fungi and many other microorganisms.[8] The best characterised PAMPs involved in the recognition of fungi are Toll-like receptors (TLR) and lectin receptors such as dectin-1.[9] The PRR-PAMP interaction triggers a cascade of complex intracellular reactions leading to the production of pro-inflammatory cytokines such as TNF, IL-1, IL-12 and IL-6 via the nuclear factor-κB (NF-κB) pathway.[10] This in turn drives CD4+ T-lymphocytes towards T helper (Th) 1 and 17 phenotype, an adaptive immune response that further augments the innate immune system.[9,11]

Susceptibility to IFD can be partly attributed to defective or ineffective cytokine production. Part of this susceptibility is genetically determined. For instance, dectin-1 polymorphism (Y238X, rs6910526) leads to a truncated carbohydrate recognition domain, which in turn leads to a defective production of IL-17, TNF, and IL-6 and is associated with significantly increased risk of IFD.[12,13] Similarly, single nucleotide polymorphisms (SNP) in other PAMPs such as TLR2 and TLR4, TLR1 and TLR5 have been shown to increase the risk of IFD through defective cytokine production.[14,15] Polymorphisms in other cytokine genes such as IL-10, IL-1, TNF receptor type 2 promoter have also been implicated as genetic biomarkers of susceptibility to IFD.[16—18] However, the prevalence of such SNPs are relatively uncommon as the vast majority of IFD patients or stem cell donors do not have any identifiable mutation. It is therefore likely that other yet unidentified mechanisms exist which affects an effective cytokine milieu.

As cytokines are key molecules that mediate and regulate effector functions of both the innate and adaptive immune systems, we hypothesise that serum cytokine levels pre-chemotherapy or HSCT (baseline cytokine profile) and levels during treatment may provide a diagnostic and prognostic value. As part of the Diagnostic and Management Strategies for Invasive Aspergillosis Study[19] we examined the role of cytokines in IFD as risk and prognostic factors by measuring 30 cytokines on sera at baseline and follow-up in a cohort of haematology patients undergoing HSCT or intensive chemotherapy. In addition, we examined nine previously reported polymorphisms associated with IFD that influence cytokine biology.

Patients and methods

Patients

Study subjects were from the Diagnostic and Management Strategies for Invasive Aspergillosis (ClinicalTrials.gov NCT00816088).[19] Only adult patients with haematological malignancy or aplastic anaemia undergoing intensive chemotherapy or HSCT with expected period of neutropenia of more than 10 days were included. Details of patient inclusion and exclusion criteria are provided elsewhere.[19] Out of the 203 recruited patients, 31 were excluded due to fever (6) or initiation of chemotherapy prior to sample collection (25) leaving 172 evaluable patients. Patients were followed for at least 120 days with median (range) follow-up of 730 (12—730) days. The diagnosis of IFD was based on the revised European Organization for Research and Treatment of Cancer (EORTC) and the Mycoses Study Group (MSG) definitions.[20] The study was approved by our local ethics committee and conducted in accordance with the Helsinki protocol (2008 revision) for medical research involving human subjects and registered with the ClinicalTrials.gov (NCT00816088).

Antifungal, chemotherapy, transplant and IST protocols

All patients received antifungal drugs according to local protocol previously described[21] and summarised here. Itraconazole solution, 200 mg twice/day was given as primary

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