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DOI:

[10.1016/j.jinf.2016.04.040](https://doi.org/10.1016/j.jinf.2016.04.040)

Document Version

Peer reviewed version

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Citation for published version (APA):

Ceesay, M. M., Kordasti, S., Rufaie, E., Lea, N., Smith, M., Wade, J., Douiri, A., Mufti, G. J., & Pagliuca, A. (2016). Baseline Cytokine profiling identifies novel risk factors for invasive fungal disease among haematology patients undergoing intensive chemotherapy or haematopoietic stem cell transplantation. *Journal of Infection*, 73(3), 280-288. <https://doi.org/10.1016/j.jinf.2016.04.040>

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Accepted Manuscript

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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PII: S0163-4453(16)30134-7

DOI: [10.1016/j.jinf.2016.04.040](https://doi.org/10.1016/j.jinf.2016.04.040)

Reference: YJINF 3765

To appear in: *Journal of Infection*

Received Date: 13 February 2016

Revised Date: 16 April 2016

Accepted Date: 20 April 2016

Please cite this article as: Ceesay MM, Kordasti S, Rufaie E, Lea N, Smith M, Wade J, Douiri A, Mufti GJ, Pagliuca A, Baseline Cytokine profiling identifies novel risk factors for invasive fungal disease among haematology patients undergoing intensive chemotherapy or haematopoietic stem cell transplantation, *Journal of Infection* (2016), doi: 10.1016/j.jinf.2016.04.040.

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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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Word count: 2,395

Tables: 4

Supplementary information: 5 figures

Running title: Baseline cytokine profile predicts IFD

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Abstract

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-1 α were significantly higher whilst IL-4 was lower in patients with proven/probable IFD compared to those with no evidence of IFD. Baseline high 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.

Key words: Invasive fungal disease, baseline cytokines, IL2R, CCL2

Highlights

- Baseline cytokine profile appears to provide significant insight into the immunological milieu prior to immunosuppressive therapy
- Baseline IL2R and CCL2 are independent indicators of risk of developing IFD
- Follow-up cytokine profile may not provide any additional value in IFD risk

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 the 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- κ B (NF- κ B) 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 Study¹⁹ 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 described²¹ and summarised here. Itraconazole solution, 200 mg twice/day was given as primary prophylaxis to high-risk patients (allogeneic HSCT, AML/MDS and salvage lymphoma chemotherapies). Umbilical cord allogeneic HSCT recipients were given posaconazole suspension 200 mg three times/day. Autologous HSCT recipients received oral fluconazole 200 mg daily. Voriconazole 200 mg twice/day was used as secondary prophylaxis. Prophylaxis was initiated at admission and continued until unsupported neutrophils were $\geq 1.0 \times 10^9/L$ for two consecutive days

and immunosuppression weaned off with no evidence of graft versus host disease (GVHD) in the case of allogeneic HSCT recipients. First-line empirical antifungal therapy was liposomal amphotericin B (3 mg/kg/day) while voriconazole was used for the treatment of proven/ probable IFD.

Transplant regimens were fludarabine-based with T-cell depletion using either antithymocyte globulin (ATG) or alemtuzumab.²² Immunosuppressive therapy for aplastic anaemia used the ATG/cyclosporine protocol.²³ Chemotherapy regimens used were disease dependent and agreed by a multi-disciplinary team.

Cytokine profiling

Thirty cytokines (EGF, EOTAXIN, FGF, G-CSF, GM-CSF, HGF, INF- α , IFN-gamma, IL-1RA, IL-1 β , IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p40/p70, IL-13, IL-15, IL-17, IP-10, CCL2, MIG, MIP-1 α , MIP-1 β , RANTES, TNF- α and VEGF) were measured using Cytokine 30-plex antibody bead kit (Life technologies, Grand Island, NY, USA) using Luminex 200TM (Luminex Corporation, Austin, TX, USA) according to the manufacturer's instructions. Serum concentrations of each cytokine were calculated from a control standard curve. The cytokine assays were performed on sera (transported on ice and freshly separated and stored at -80°C until analysis) at baseline (before initiation of therapy or fever) and fortnightly during inpatient admission.

Genetic polymorphisms

Nine single-nucleotide polymorphisms (SNP) were analysed using TaqMan SNP Genotyping Assays (Applied Biosystems). Analysis was performed from genomic DNA isolated from whole peripheral blood. The following genes / SNP's (dbSNP ref ID) were analysed TLR1 (rs5743611), TLR2 (rs5743708), TLR3 (rs3775291), TLR4.1 (rs4986791), TLR4.2 (rs4986790), TLR6 (rs5743810), CLEC7A

(rs16910526), CARD9 (rs4077515), and INFG (rs2069705). Samples from 189 patients (83 allogeneic HSCT recipients, 62 allogeneic donors, and 44 autologous HSCT recipients) were genotyped. Genotyping was carried out as described previously.^{13-15,24-27}

Statistical analysis

Data on baseline serum cytokine concentrations were presented as median (IQR) and compared between patients with proven/probable IFD and those with no evidence of IFD using the Mann-Whitney U test. Cox proportional hazards models were used to investigate the role of baseline cytokine concentrations as potential predictors of IFD. All variables examined in the univariate model with $P < 0.10$ were entered in the multivariate model, described elsewhere¹⁹ and included relevant cytokines, haematological diagnoses, index haematological treatment, bacteraemia during the study period, monocytopenia > 10 days, Karnofsky score, and baseline CT scan. Significance was assessed by forward stepwise method based on Likelihood Ratios. Adjusted hazard ratios (HR) and 95% CI were calculated. Separate models were created for prognostic factors. The best fit for final model was with IFD and not IA.

Follow-up cytokines were compared graphically by plotting the mean of log transformed value per time point, with error bars indicating the 95% CI, according to outcome (proven/probable v. evidence of IFD). Patients with possible IFD and not classified were excluded from this analysis due to uncertainty of outcome.

The analyses were performed using SPSS statistics version 20 (IBM Corporation, New York, USA). P values < 0.05 were considered statistically significant.

Results

Baseline cytokines

Baseline serum cytokine concentrations were available for 172 patients (Table 1). The final EORTC/MSG classifications were: proven *Aspergillus fumigatus* (2) mould NOS (1), *Fusarium* spp. (1), *Candida* spp. (4), probable IFD (21), possible IFD (14), not classified (67), and no evidence of IFD (62). The incidence of proven/probable IFD and IA in this cohort was therefore 16.9 % (29/172) and 14.4 % (24/167), respectively. Co-infection with bacteraemia occurred in two patients; *Micrococcus* spp. in an allogeneic HSCT recipient with probable IFD and Vancomycin-resistant *enterococcus* in a chemotherapy recipient with proven *Aspergillus fumigatus*. There were four additional patients with bacteraemia who had unclassified IFD but none in those with no evidence of IFD. Twelve patients developed proven/probable IFD (*Aspergillus fumigatus* [1], *Fusarium* spp. [1], *Candida* spp. [2], and probable IFD [8]) among the allogeneic HSCT patients giving an IFD incidence of 16.9 % (12/71). This reflects the incidence in the larger cohort of the study population previously described.¹⁹

Five cytokines were found to be significantly different between patients with proven/probable IFD compared to those with no evidence of IFD. Median concentrations of IL-15, IL-2R, CCL2, and MIP-1 α were higher while IL-4 was lower in patients with proven/probable IFD compared to those with no evidence of IFD (Table 2).

The role of baseline cytokines as risk factors for IFD was assessed using Cox proportional hazard model previously described.¹⁹ The median (IQR) CCL2 and IL-2R concentrations were 419.0 pg/ml (150.2-840.6 pg/ml) and 443.8 pg/ml (297.0-

833.6 pg/ml) respectively. Among the 19 patients with CCL2 above the third quartile, 13 (68.4%) developed IFD compared to 16 of the 72 patients (22.2%) with IL-2R \leq first quartile (Pearson $\chi^2 = 14.78$, $P < 0.001$). The adjusted Hazard ratio (HR) for IFD in patients with CCL2 $>IQR$ was 2.7 (95% CI 1.2-6.1; $P = 0.016$). Of the 24 patients with IL-2R $>IQR$, 13 (54.2%) developed IFD compared to 16 of the 67 patients (23.9%) with serum concentrations $\leq IQR$ (Pearson $\chi^2 = 7.47$, $P = 0.006$). The adjusted HR for IFD in those with IL-2R $>IQR$ was 2.3 (95% CI 1.1-5.1; $P = 0.037$). The adjusted HR (95% CI) for low IL-4, high MIP-1 α , and IL-15 were 2.5 (0.9 – 7.2; $P = 0.090$), 1.7 (0.7 – 4.2; $P = 0.279$), and 1.2 (0.5 – 2.9; $P = 0.759$), respectively. The multivariate model showed that high CCL2 and IL-2R at baseline were independent risk factors for developing IFD.

Follow-up cytokines

A total of 412 serum follow-up samples from the 172 patients who had evaluable baseline patients were analysed. The median number of follow up cytokine samples per patient was 2 (range 2-15). Overall no significant differences were detected between patients with IFD compared to those with no evidence of IFD (Supplementary Figures S1-S5). However, IL-2R was consistently higher in IFD patients and appeared to increase with longer follow up (Figure S3).

Genetic polymorphisms

A total of 127 patients (median age 53 [range 19-73] years, 76 [60%] male, 83 allografts and 44 autografts, 111 [87%] white Europeans) were tested for nine SNPs. In addition, 62 (67%) donors of allogeneic HSCT were also tested. Among these 127 patients 25 were proven/probable IFD, 20 proven/probable IA, 15 possible IFD, 48 not classified, and 39 had no evidence of IFD. No statistically significant differences in any of the genetic polymorphisms were found between IFD cases

compared to those with no evidence of IFD (Table 3). Among donors of allogeneic HSCT, no SNP was found in TLR 4.2 (rs4986790, A→G D299G) among patients with IFD compared to 4 (36.4%) cases in those with no IFD (Table 4).

Prognostic factors

Baseline cytokine serum levels were assessed as potential prognostic factors using the Cox regression model. A univariate model was created for the 30 cytokines. High IL-13 and IL-6 were associated with increased risk of death with odds ratios of 1.8 (95% CI 1.0-3.4) and 2.4 (95% CI 1.3-4.3) respectively. A multivariate model was then created which included IL-6, IL-13, ITU admission, IFD (proven/probable v no evidence of IFD), hepatic dysfunction, renal dysfunction (creatinine > 240 umol/L), bacteraemia, underlying haematological diagnosis and treatment, which were shown to be significant from the larger cohort. ITU admission, IFD diagnosis, index haematological treatment, and renal dysfunction, were significant independent prognostic factors. The associated adjusted HR (95% CI) for death were 3.11 (1.03 - 9.43, P = 0.045), 2.57 (1.03 - 6.40, P = 0.042), 4.51 (0.97 - 21.03, P = 0.055), and 21.74 (4.59 - 100.00, P = 0.011), respectively. Neither IL-6 nor IL-13 had any significant independent prognostic value.

Discussion

Cytokines and chemokines, produced by an array of immune cells, form an important component of host defences against infection by various pathogens. Soluble IL-2R, produced primarily by activated T-cells, is a marker of antigenic stimulation but high baseline levels may represent an ineffective adaptive immune response.²⁸ CCL2 is an important chemo-attractant for monocytes and dendritic cells especially in

neutropenic setting.²⁹ In this study we demonstrated the usefulness of measuring baseline cytokines from peripheral blood (minimally invasive and convenient) as independent risk factors for IFD.

IFD is a disease of the immunocompromised and many risk factors have been described.³⁰⁻³² Recently we described additional risk factors such as baseline CT abnormalities, prolonged monocytopenia, and poor performance status.¹⁹ IL-2R and CCL2 at baseline are novel signalling pathways indicating risk of IFD.

IL-2 is produced primarily by activated CD4+ T-cells and activates a number of key cells in the immune system in both autocrine (stimulation and expansion of antigen-specific T cells) and paracrine (effect on B cells, natural killer cells, neutrophils, monocytes) manner via the IL-2R.²⁸ Soluble IL-2R is a truncated form of the IL-2R α subunit but retains the ability to bind IL-2 efficiently.^{33,34} Elevated sIL-2R levels have been found in autoimmune diseases, malignancies especially adult T-cell leukaemia/lymphoma and hairy cell leukaemia, and infections such as tuberculosis and brucellosis.^{28,35} The biological role of sIL-2R in all these conditions as well as in IFD is likely to be one of immunosuppression through competitive binding of IL-2 and thus curtailing an effective adaptive immune response against the fungus or the malignant clone. The competitive binding of IL-2 by sIL-2R may explain the lack of difference in our cohort between patients with proven/probable IFD and those with no evidence of IFD in our cohort.

CCL2 was first identified as a monocyte chemoattractant and was initially named monocyte chemoattractant protein-1 (MCP-1).³⁶ High levels have been linked to the pathogenesis and spread of many cancers such as prostate, breast, and colorectal

cancer.³⁷⁻³⁹ CCL2 is thought to stimulate the tumour microenvironment by a variety of mechanisms including direct stimulation of the tumour cells while preventing an effective immune response.^{40,41} This ineffective immune response is likely to underlie increased IFD in haematology patients with high baseline CCL2. This effect is independent of monocytopenia, which occurs during follow-up after the administration of chemotherapy or immunosuppressive therapy.

It could be argued that the cytokine milieu that predict IFD is non-specific for fungal pathogens as the signaling pathway involved in cytokine biology is shared with other micro-organisms such as bacteria. However, the limited number of bacteraemias (6 cases) in this study makes it difficult to assess the role of cytokines in bacterial sepsis especially as these were co-infections. Larger studies involving greater numbers of bacteraemia cases will be required to address this association.

Follow-up cytokine assays were performed on 412 samples with a median of 2 (range 2-15) samples per patient collected on a fortnightly basis. This skew to the left is a reflection of the fewer measurements being available in later time points as patients engraft and get discharged from hospital. No significant differences were apparent between patients with proven/probable IFD compared to those with no evidence of IFD. More frequent sampling may have detected a pattern of cytokines that differentiate between IFD v no evidence of IFD but this will require further evaluation. The lack of difference may also reflect the fact that cytokines are mainly an innate immune response and as such lack specificity for fungal infection at a time when patients may be having additional inflammatory challenges from chemotherapy, donor cell infusion, as well as fever from non-IFD cases. An additional explanation may be the fact that the ineffective immune system that

enabled IFD at baseline such as the high levels of the immunosuppressant sIL-2R may no longer be relevant during follow-up.

Chai et al investigated 119 patients with IA from the Global Comparative Aspergillosis Study⁴² and found high baseline IL-8 and persistently elevated IL-6 and CRP as poor prognostic factors.⁴³ Important differences exist between that study and the current one. Firstly, the Global Comparative Aspergillosis Study was a clinical trial comparing voriconazole v amphotericin B and the case definitions for proven and probable IA were based on earlier EORTC/MSG 2002 definitions.⁴⁴ Secondly, there was no comparator arm in that trial as the study entry criterion was proven/probable IA. It is therefore difficult to assign the cytokine dynamics to IA. Thirdly, the baseline values in that study refers to IA prior to initiation of antifungal therapy, which is in contrast to true baseline values in the current study where the patients showed no clinical or biochemical signs of sepsis and prior to chemotherapy, transplant or IST. Finally, the persistently high CRP and IL-6 after initiation of antifungal therapy may mean that the affected patients were not responding to therapy. This is especially relevant in a disease that is difficult to diagnose and the majority of patients were probable cases, some of which would be unclassified on the revised EORTC/MSG definitions.

Genetic predisposition to IFD has generated a lot of interest recently.^{12-14,18,45-47} The selection of nine genes to examine for this susceptibility was based on these studies. No statistically significant associations were found between these previously described SNPs and susceptibility to IFD in our cohort. The reason for this is likely to be due to sample size and/or the small number of IFD cases in the cohort.

In conclusion, we have demonstrated that high baseline IL-2R and CCL2 are independent indicators of the risk of IFD. High serum levels may reflect ineffective immune environment, which in concert with further immunosuppression during haematological treatment could lead to increased incidence of IFD. Although we used the 30-plex approach, the 2 cytokines can be incorporated into routine biochemistry laboratories.

Author contribution

MMC designed the study, performed cytokine assays, analysed data, and wrote manuscript. SK performed cytokine assays and reviewed manuscript. ER and NL conducted the SNP work and reviewed manuscript. MS and JW designed the study and reviewed manuscript. AD provided statistical support and reviewed manuscript. GJM designed the study and reviewed manuscript. AP designed the study, oversaw study conduct, and reviewed manuscript.

Acknowledgments: The authors would like to thank the KCL Tissue Bank for storing and retrieving study samples and Bolaji Coker for data management.

Funding: This work was supported by an unrestricted educational grant from Gilead Sciences and Pfizer.

Conflict of interest

None

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Table 1: Baseline demographic and clinical characteristics of study patients

Characteristic	Patients N = 172
Age, median (range)- years	54 (19 - 74)
Sex – n (%)	
Males	102 (59)
Females	70 (41)
Underlying disease	
MDS/AML	69 (40)
Non-Hodgkin lymphoma	29 (17)
Multiple myeloma	43 (25)
Aplastic anaemia	17 (10)
Acute Lymphoblastic Leukaemia	4 (2)
Hodgkin lymphoma	4 (2)
Myeloproliferative neoplasms	6 (4)
Haematological treatment	
Allogeneic HSCT	71 (41)
Autologous HSCT	62 (36)
Chemotherapy - only	30 (18)
Immunosuppressive therapy - only	9 (5)
EORTC/MSG classification	
Proven mould	4 (2)
Proven candida	4 (2)
Probable IFD	21 (12)
Possible IFD	14 (8)
Not classified	67 (39)
No evidence of IFD	62 (36)

Table 2: Baseline cytokine profile

Cytokine	IFD N= 29		no IFD N=62		Mann-Whitney p-value
	Median	IQR	Median	IQR	
EGF	65.1	21.6-208.4	82.4	29.5-218.1	0.285
EOTAXIN	74.7	31.0-150.0	63.4	37.8-100.6	0.740
FGF	14.6	5.5-27.4	13.0	9.2-22.5	0.692
GCSF	69.0	29.4-267.1	43.1	25.2-231.0	0.450
GMCSF	20.4	20.3-24.1	20.4	19.7-24.1	0.696
HGF	400.2	176.7-1346.1	179.2	67.9-829.8	0.071
IFN- α	27.7	20.3-48.1	23.6	19.1-34.3	0.259
IFN- γ	19.6	17.3-39.2	19.3	17.3-37.7	0.562
IL-10	8.4	6.0-10.9	9.4	5.8-13.1	0.762
IL-12	112.4	76.7-260.1	146.1	87.1-213.7	0.512
IL-13	27.4	25.7-49.4	25.7	20.7-35.6	0.223
IL-15	29.5	19.2-64.9	20.9	7.4-29.5	0.035
IL-17	16.9	10.6-16.9	16.9	11.8-17.1	0.243
IL-1 β	16.9	9.8-19.2	14.1	9.8-16.9	0.303
IL-1RA	362.7	167.4-696.2	266.3	192.2-640.2	0.851
IL-2	6.7	4.8-13.7	6.4	5.0-9.9	0.746
IL-2R	616.7	322.3-1208.9	411.3	284.1-730.8	0.039
IL-4	9.0	4.0-36.1	33.8	9.0-51.6	0.010
IL-5	7.0	2.6-7.0	7.0	3.4-7.0	0.632
IL-6	7.9	3.4-30.7	7.9	4.4-15.3	0.645
IL-7	15.1	6.7-27.3	24.9	6.5-28.9	0.746
IL-8	86.9	51.6-470.2	64.2	27.3-284.5	0.193
IP-10	44.1	23.2-169.3	43.9	26.1-75.1	0.676
CCL2	785.8	353.2-1421.8	333.5	144.9-620.8	0.003
MIG	85.9	43.2-168.5	85.9	52.4-182.2	0.925
MIP-1 α	37.2	25.2-125.0	28.6	20.2-48.1	0.049
MIP- β	70.5	37.0-136.0	65.2	44.9-133.2	0.939
RANTES	3609.6	600.7-7622.6	6510.0	4958.2-8300.0	0.074
TNF- α	4.8	4.0-7.3	4.6	2.3-7.3	0.426
VEGF	15.9	3.7-22.0	22.0	3.6-29.8	0.453

Table 3: Association between genetic polymorphisms and IFD in patients

	IFD	No IFD	P value
TLR1 N=63			
Major allele (C/C) n=57	21 (84.0)	36 (94.7)	0.182
Minor allele (G/G) n=2	2 (8.0)	0	
Major/Minor (C/G) n=4	2 (8.0)	2 (5.3)	
Any SNP (C/G or G/G) n=6	4 (16.0)	2 (5.3)	0.156
TLR2 n=58			
Major allele (G/G) n=50	18 (81.8)	32 (88.9)	0.449
Minor allele (A/A) n=0	0	0	
Major/Minor G/A n=8	4 (18.2)	4 (11.1)	
Any SNP (G/A, A/A) n=8	4 (18.2)	4 (11.1)	0.449
TLR3 n=63			
Major allele (C/C) n=40	17 (68.0)	23 (60.5)	0.349
Minor allele (T/T) n=3	0	3 (7.9)	
Major/Minor (C/T) n=20	8 (32.0)	12 (31.6)	
Any SNP (C/T, T/T) n=23	8 (32.0)	15 (39.5)	0.547
TLR4.1 n=62			
Major allele (C/C) n=54	22 (88.0)	32 (86.5)	0.862
Minor allele (T/T) n=0	0	0	
Major/Minor (C/T) n=8	3 (12.0)	5 (13.5)	
Any SNP (C/T or T/T) n=8	3 (12.0)	5 (13.5)	0.862
TLR4.2 n=60			
Major allele (A/A) n=52	18 (81.8)	34 (89.5)	0.401
Minor allele (G/G) n=0	0	0	
Major/Minor (A/G) n=8	4 (18.2)	4 (10.5)	
Any SNP (G/G or A/G) n=8	4 (18.2)	4 (10.5)	0.401
TLR6 n=61			
Major allele (G/G) n=29	13 (54.2)	16 (43.2)	0.484
Minor allele (A/A) n=6	3 (12.5)	3 (8.1)	
Major/Minor (G/A) n=26	8 (33.3)	18 (48.6)	
Any SNP (G/A or A/A) n=32	11 (45.8)	21 (56.8)	0.404
CLEC7A (dectin-1) n=61			
Major allele (A/A) n=51	20 (83.3)	31 (83.8)	0.963
Minor allele (C/C) n=0	0	0	
Major/Minor (A/C) n=10	4 (16.7)	6 (12.2)	
Any SNP (A/C or C/C) n=10	4 (16.7)	6 (12.2)	0.963
CARD9 n=60			
Major allele (C/C) n=15	7 (31.8)	8 (21.1)	0.183
Minor allele (T/T) n=13	2 (9.1)	11 (28.9)	
Major/Minor (G/T) n=32	13 (59.1)	19 (50.0)	
Any SNP (G/T or T/T) n=45	15 (68.2)	30 (78.9)	0.353
INFg n=64			
Major allele (G/G) n=5	3 (12.0)	2 (5.1)	0.566
Minor allele (A/A) n=26	9 (36.0)	17 (43.6)	
Major/Minor (G/A) n=33	13 (52.0)	20 (51.3)	
Any SNP (G/A or A/A) n=59	22 (88.0)	37 (94.9)	0.318

Table 4: Association between genetic polymorphisms and IFD in allogeneic HSCT donors

	IFD	No IFD	P value
TLR1 N=29			
Major allele (C/C) n=25	15 (93.8)	10 (76.9)	0.191
Minor allele (G/G) n=0	0	0	
Major/Minor (C/G) n=4	1 (6.2)	3 (23.1)	
Any SNP (C/G or G/C) n= 4	1 (6.2)	5 (23.1)	0.191
TLR2 n=28			
Major allele (G/G) n=27	15 (100)	12 (92.3)	0.274
Minor allele (A/A) n=0	0	0	
Major/Minor G/A n=1	0	1 (7.7)	
Any SNP (G/A, A/A) n=1	0	1 (5.6)	0.274
TLR3 n=26			
Major allele (C/C) n=14	6 (42.9)	8 (66.7)	0.375
Minor allele (T/T) n=1	1 (7.1)	0	
Major/Minor (C/T) n=11	7 (50)	4 (33.3)	
Any SNP (C/T, T/T) n=12	8 (57.1)	4 (33.3)	0.225
TLR4.1 n=28			
Major allele (C/C) n=23	14 (93.3)	9 (69.2)	0.228
Minor allele (T/T) n=1	0	1 (7.7)	
Major/Minor (C/T) n=4	1 (6.7)	3 (23.1)	
Any SNP (C/T or T/T) n=5	1 (6.7)	4 (30.8)	0.097
TLR4.2 n=24			
Major allele (A/A) n=20	13 (100)	7 (63.6)	0.059
Minor allele (G/G) n=1	0	1 (9.1)	
Major/Minor (A/G) n=3	0	3 (27.3)	
Any SNP (G/G or A/G) n=4	0	4 (36.4)	0.017
TLR6 n=26			
Major allele (G/G) n=13	9 (60)	4 (36.4)	0.188
Minor allele (A/A) n=6	4 (26.7)	2 (18.2)	
Major/Minor (G/A) n=7	2 (13.3)	5 (45.5)	
Any SNP (G/A or A/A) n=13	6 (40)	7 (63.6)	0.234
CLEC7A (dectin-1) n=31			
Major allele (A/A) n=27	13 (92.9)	14 (82.4)	0.414
Minor allele (C/C) n=2	0	2 (11.8)	
Major/Minor (A/C) n=2	1 (7.1)	1 (5.9)	
Any SNP (A/C or C/C) n=4	1 (7.1)	3 (17.6)	0.385
CARD9 n=26			
Major allele (C/C) n=12	6 (40)	6 (54.5)	0.760
Minor allele (T/T) n=3	2 (13.3)	1 (9.1)	
Major/Minor (G/T) n=11	7 (46.7)	4 (36.4)	
Any SNP (G/T or T/T) n=14	9 (60)	5 (45.5)	0.462
INFg n=25			
Major allele (G/G) n=6	2 (16.7)	4 (30.8)	0.618
Minor allele (A/A) n=3	2 (16.7)	1 (7.7)	
Major/Minor (G/A) n=16	8 (66.7)	8 (61.5)	
Any SNP (G/A or A/G) n=19	10 (83.3)	9 (69.2)	0.409

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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Highlights

- Baseline cytokine profile appears to provide significant insight into the immunological milieu prior to immunosuppressive therapy
- Baseline IL2R and CCL2 are independent indicators of risk of developing IFD
- Follow-up cytokine profile may not provide any additional value in IFD risk