A Prospective Observational Multi-institutional Study on Invasive Fungal Infections Following Chemotherapy for Acute Myeloid Leukemia (MISFIC Study): A Real World Scenario from India

Biju George¹ · Hari Menon² · Dinesh Bhurani³ · Sharat Damodar⁴ · Shashi Apte⁵ · Tulika Seth⁶ · Ajay Sharma⁷ · Radhe Shyam⁸ · Pankaj Malhotra⁹ · Jose Easow¹⁰ · Kavitha M. Lakshmi¹ · Narendra Agrawal³ · Manju Sengar² · KS Nataraj⁴ · Rayaz Ahmed³ · Sanjeevan Sharma⁷ · Alka Khadwal⁹ · Gaurav Prakash⁹ · Aby Abraham¹ · Anup Devasia¹ · Anu Korula¹ · Vikram Mathews¹

Received: 18 July 2019 / Accepted: 13 August 2019 / Published online: 26 August 2019
© Indian Society of Hematology and Blood Transfusion 2019

Abstract We performed a prospective multi-centre observational study to understand the incidence of IFI in patients with AML in India with use of anti-fungal prophylaxis. All patients with AML receiving either induction chemotherapy or salvage chemotherapy between November 2014 and February 2016 were included in this prospective observational study from 10 Indian centres. IFI was defined as per the revised EORTC-MSG criteria. Data on type of chemotherapy used, type of anti-fungal prophylaxis used, time to neutrophil recovery, incidence of IFI and survival were collected. Two hundred patients (118 male and 82 females) with a median age of 35 years (range: 2–66) were recruited. One hundred and eighty-six (93%) had newly diagnosed acute myeloid leukemia (AML) while 14 (7%) had relapsed disease. IFI occurred in

53 patients (26.5%) with proven or probable IFI occurring in 17 (8.5%). Use of posaconazole prophylaxis ($p = 0.027$) was the only factor found to be associated with a reduced incidence of IFI. The overall survival (OS) at 6 weeks and 3 months respectively was similar among patients who had IFI ($83.0 \pm 5.2\%$; $81.0 \pm 5.4\%$) as compared to those without IFI ($84.4 \pm 3.0\%$; $81.4 \pm 3.2\%$). This prospective study reveals a high incidence of IFI in patients undergoing chemotherapy for AML in India. The use of posaconazole prophylaxis was associated with a significantly lower incidence of IFI. Optimal strategies to prevent IFI need to be studied.

Keywords Fungal infection · Incidence · AML · India

Introduction

Invasive fungal infections (IFI) are a major cause of morbidity and mortality following induction or salvage chemotherapy for acute myeloid leukaemia (AML) with reported incidences of all category IFIs varying between 2 and 49% with higher rates reported from Asia [1–8]. Both anti-fungal prophylaxis and empirical therapy with anti-fungal agents have played an important role in reducing the incidence of IFIs in patients undergoing chemotherapy for AML [9–12]. Therefore the standard recommendations from a number of infectious disease (ID) societies is to use primary prophylaxis with antifungal drugs usually posaconazole during induction or salvage chemotherapy for AML [13–15]. Though carefully controlled randomized controlled trials have shown reduction in the incidence of IFI, it is important to see whether the same results are

✉ Biju George
Biju@cmcvellore.ac.in

¹ Christian Medical College, Vellore, India

² Tata Memorial Hospital, Mumbai, India

³ Rajiv Gandhi Cancer Institute, New Delhi, India

⁴ Narayana Hirudalaya, Bengaluru, India

⁵ Sahayadri Hospital, Pune, India

⁶ All India Institute of Medical Sciences, New Delhi, India

⁷ R and R Hospital, New Delhi, India

⁸ HCG Hospital, Bengaluru, India

⁹ Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India

¹⁰ Apollo Specialty Hospital, Chennai, India

obtained in the real world setting and higher incidences have been reported in other studies from India [8, 16, 17]. It was therefore proposed to perform a prospective observational trial to document the incidence of IFI in patients undergoing chemotherapy for AML in the presence of anti-fungal prophylaxis.

Patients and Methods

This is a prospective multi-institutional study on the incidence of fungal infections following chemotherapy for acute myeloid leukemia (MISFIC study). All consecutive consenting patients who underwent induction or salvage chemotherapy for acute myeloid leukaemia (AML) except for AML-M3 between November 2014 and February 2016 in 10 participating institutions were analyzed. This study was approved by the local Institutional Review Board at each of the participating centres. Patients having induction chemotherapy with hypomethylating agents or having consolidation therapy with High dose cytarabine while in remission were excluded. Patients who had pre-existing fungal infection or those who developed IFI within 72 h of initiation of chemotherapy were also excluded. Data collected from individual medical records included patient demographics, type of chemotherapy used, type of anti-fungal prophylaxis used, time period to resolution of neutropenia, incidence, site and treatment of fungal infection and overall survival at 3 months.

Management of Neutropenia and Sepsis

This was an observational study and hence each institution was allowed to follow their individual institutional protocols on management of febrile neutropenia. Patients generally underwent chemotherapy in non-HEPA filtered rooms. No antibacterial or anti-viral prophylaxis was administered during chemotherapy. The choice of the antifungal agent for anti-fungal prophylaxis was left to each institution. The type of antibiotic that was used for initial therapy of febrile neutropenia was also according to institutional protocols of each centre. In the presence of persistent fever for > 72 h despite adequate antibacterial therapy, High resolution CT scans (HRCT) of the chest were performed. In patients with suspicious findings on the HRCT, serum galactomannan levels were sent (in centres having this facility to perform this test). All centres had access within their own institution to a broncho-alveolar lavage (BAL) being performed but that decision was left to the individual physician. The decision on the change of antifungal drugs was at the discretion of the individual treating physician.

Diagnosis and Management of Fungal Infection

If there was a clinical suspicion of fungal infection (persistent fever > 72 h while on appropriate antibiotic therapy), patients had a high resolution CT scan (HRCT) of the chest performed, if clinically possible. If there was a lung infiltrate seen on HRCT suggestive of fungal infection, attempts were made to obtain cultures for diagnosis including sputum cultures, bronchoscopy with broncho-alveolar lavage and biopsy depending upon the clinical condition of the patient. Galactomannan testing for fungal antigen on peripheral blood was performed if the test was available within the treating institution. Fungal infections were defined as per the modified EORTC-MSG criteria [18]. In patients with suspected IFI, the local physician was allowed to change antifungal drugs used for prophylaxis based on the local institutional practice.

Statistical Analysis

The primary objective of the study was to evaluate the incidence of breakthrough invasive fungal infection (IFI) in patients undergoing intensive chemotherapy for AML. Secondary objectives included identifying potential risk factors for the development of IFIs and mortality related to fungal infection. Multivariate analysis using the Cox regression analysis was done to look for variables that influenced the incidence of IFI. Statistical analyses were performed with SPSS (windows 16 version, SPSS Inc, Chicago). For all variables, a two-sided *p* value of 0.05 or less was considered statistically significant.

Results

Between November 2014 and February 2016, 208 patients were recruited from 10 participating institutions of which 8 patients were excluded since they were deemed to have fungal infection at the time of diagnosis of AML. Baseline characteristics for the 200 patients are shown in Table 1. There were 118 male and 82 females with a median age of 35 years (range: 2–66). One hundred and eighty-six (93%) had newly diagnosed acute myeloid leukemia (AML) while 14 (7%) had relapsed disease. All patients received intensive chemotherapy while patients receiving treatment with azacytidine were excluded from this study. Majority of the patients received chemotherapy in single or double rooms and not in HEPA filtered rooms. All patients received anti-fungal prophylaxis with majority (89%) receiving posaconazole while others received voriconazole, amphotericin B or fluconazole as per local institutional practices.

Table 1 Baseline characteristics of patients with AML undergoing chemotherapy

Variables	Median (range)/number (%)
Total number of patients	200
Age (years)	35 (2–66)
Sex	
Male	118 (59%)
Female	82 (41%)
Status of disease	
Newly diagnosed	186 (93%)
Relapsed disease	14 (7%)
Chemotherapy regimens	
Cytosine/daunorubicin (7/3)	161 (80.5%)
Cytosine/daunorubicin (5/2)	17 (8.5%)
Paediatric BFM	8 (4%)
High dose cytosine, Flag, HAM, others	14 (7%)
Type of antifungal prophylaxis	
Posaconazole	178 (89%)
Others (amphotericin B, voriconazole, fluconazole)	22 (11%)

Response to Chemotherapy

Of the 200 patients who received chemotherapy, 128 (64%) achieved remission while 37 (18.5%) showed evidence of residual disease on evaluation between days 28–40 following chemotherapy and 15 (17.5%) expired without evaluation of remission.

Incidence and Management of Fungal Infection

All patients had a CT scan of the chest/other organs done if clinically indicated. Galactomannan testing was available in 7 out of the 10 participating centres. Invasive fungal infection (IFI) was detected in 53 patients (26.5%) at a median of 16.1 days (range: 6–40) following initiation of chemotherapy. This included proven infection in 1 (0.5%), probable infection in 16 (8%) and possible infection in 36 patients (18%). The overall incidence of proven and probable infection was 8.5%. The main sites of fungal infection (proven/probable) included the lungs in 12 patients, sinuses and gastro-intestinal tract in 2 each while the only proven infection was candida tropicalis in the blood (Table 2). Even among patients with

possible fungal infection, lungs were the predominant focus of infection. The overall incidence of IFI was lower in patients having posaconazole prophylaxis (overall IFI—24.1%; Proven/probable—6.1%) compared to the use of other anti-fungal drugs (overall IFI—42.3%; Proven/probable—27.2%) ($p = 0.05$). Anti-fungal drugs were changed in 76 patients which included change for the treatment of IFI in 46 patients (30 on posaconazole and 16 on other anti-fungal drugs) while the same anti-fungal prophylaxis (posaconazole in all) was continued in 7 patients with possible fungal infection. Newer anti-fungal agents instituted for the treatment of IFI included liposomal amphotericin in 20, Amphotericin B desoxycholate in 13, Caspofungin in 7 and Voriconazole in 6. Among patients who had either proven or probable IFI, anti-fungal drugs were changed to liposomal amphotericin in 9, Amphotericin B desoxycholate in 6 and Caspofungin in 2.

Other Infections

Bacterial infections occurred in 76 patients (38%) with 87 episodes of bacteremia. Organisms were predominantly

Table 2 Sites of invasive fungal infection in 200 patients with AML

Sites	Number (%)	IFI as per EORTC criteria	Organism/antigen
Lungs alone	42 (79.2%)	Probable—13; Possible—29	GM + in 13
GIT	4 (7.5%)	Probable—2; Possible—2	GM + in 2
Sinuses	2 (3.8%)	Probable—2	GM + in 2
Emperic	3 (5.7%)	Possible—3	
Skin	1 (1.9%)	Possible—1	
Blood	1 (1.9%)	Proven—1	Candida tropicalis

gram negative (63/87) with many infections occurring due to carbapenem resistant organisms (mainly *Klebsiella*, *Escherichia coli* and *Pseudomonas aeruginosa*) while 24 were due to gram positive organisms (24/87)—mainly *Staphylococcus aureus* and *Enterococcus faecalis*. The incidence of IFI in patients with bacterial infection (31.5%) was not significantly different from those without a bacterial infection (23.3%; $p = 0.248$).

Predictors of Fungal Infection and Outcomes of IFI

A number of variables were analysed to look for predictive factors for breakthrough IFI in patients having anti-fungal prophylaxis. Logistic regression analysis identified female sex and use of posaconazole prophylaxis as protective against IFI while on a multivariate analysis, only the use of posaconazole prophylaxis continued to remain significant (Table 3). The 6 week and 3 month overall survival (OS) was not significantly different between patients with an IFI ($83.0 \pm 5.2\%$; $81.0 \pm 5.4\%$) and patients without IFI ($84.4 \pm 3.0\%$; $81.4 \pm 3.2\%$) (Fig. 1). The 3 month OS for patients with proven/probable IFI was lower ($73.3 \pm 1.1\%$) compared to patients with possible IFI ($84.1 \pm 5.9\%$) and no IFI ($81.4 \pm 3.2\%$) but differences were not statistically different ($p = 0.692$) (Fig. 2).

Discussion

Invasive fungal infections (IFI) have always remained one of the major complications following intensive chemotherapy for acute myeloid leukaemia mainly related to the period of neutropenia that follows such treatment. Randomized controlled studies have shown the benefit of

anti-fungal prophylaxis in reducing the incidence of IFI especially with the use of posaconazole [9–11]. The reported incidence of IFI in developing countries is higher than incidences reported from developed countries which may partly be related to administration of chemotherapy and continued care of patients with AML in non-HEPA filtered rooms along with construction work that usually happens round the year in many hospitals [6, 8, 16]. It is in this context that this prospective observational study was performed to understand the incidence of IFI in a “real world” situation with the use of antifungal prophylaxis. All participating centres were allowed to continue their own institutional practices with regard to the use of antifungal drugs for prophylaxis and also the drugs used in treating breakthrough IFI.

The overall incidence of IFI was high at 26.5% with an incidence of Proven/probable IFI being 8.25%. A meta-analysis involving 49 published studies suggested a IA risk of 4% with the use of anti-fungal prophylaxis but real world data report incidences ranging from 1.7 to 12% despite the use of antifungal prophylaxis [19–21]. The actual incidence in our study may have been even higher since few centres did not have access to the use of galactomannan antigen during the period of this study and therefore all IFIs from those centres were reported as possible IFI. Most of the IFI occurred commonly in the lungs and the sinuses as has been described in other series. The incidence of IFI was significantly lower with the use of posaconazole as compared to other antifungal drugs as has been previously reported; however we could not compare individual drugs with posaconazole due to lack of numbers. Even with the use of posaconazole, the incidence of proven/probable IFI is still high at 6% which probably reflects the increased risk associated with treatment in non-HEPA

Table 3 Logistic regression analysis studying factors influencing invasive fungal infection (IFI) in AML

Variables	Univariate analysis		Multivariate analysis	
	HR	<i>p</i> value	HR	<i>p</i> value
Age in years				
≤ 15 versus > 15	1.024 (0.381–2.753)	0.967	–	–
Days to ANC > 500	1.042 (0.997–1.089)	0.069	1.042 (0.995–1.091)	0.079
Sex				
Male versus female	0.417 (0.209–0.833)	0.013	0.514 (0.238–1.110)	0.090
Status of disease				
Newly diagnosed versus relapsed disease	0.441 (0.95–2.04)	0.295	–	–
Type of chemotherapy				
Induction versus salvage		0.103	–	–
Type of prophylaxis				
Other prophylaxis versus posaconazole	3.228 (1.311–8.001)	0.011	4.312 (1.486–12.510)	0.007

Fig. 1 Comparison of overall survival (OS) between patients with IFI and No IFI

Fig. 2 Comparison of 3 month OS of patients with proven/probable IFI with those of possible IFI and no IFI

filtered rooms and the increasing construction activity that occurs round the year in most hospitals. In this study, we do not have any data on posaconazole levels in patients who were receiving posaconazole for anti-fungal prophylaxis.

Majority of the patients showed response to institution of other anti-fungal drugs when breakthrough IFI was reported with resolution of symptoms and this resulted in similar overall survival between those who had IFI and those who did not have IFI. This is in keeping with more

recent studies which seems to suggest that use of posaconazole may not impact on overall survival since better drugs are available for treatment of IFI if it occurs [22, 23]. One of the possible confounders in our series is also the fact that a number of patients develop gram negative sepsis (esp with ESBL and CRO organisms) and this has an additional significant impact on mortality in patients undergoing chemotherapy for acute myeloid leukaemia.

There has always been speculation about whether possible IFI should be considered as true IFI since in most series, it contributes to more than 60–70% of total IFI that is reported. In our series also, the incidence of possible IFI was 18.25% compared to 6.25% for proven/probable IFI. It is heartening to note that the overall survival of patients with possible IFI were higher than those with proven/probable IFI and similar to the OS with patients with no IFI suggesting that many of these patients may not have had IFI at all. In areas with a high burden of fungal disease such as India, a single strategy may not be ideal and therefore a combination of anti-fungal prophylaxis along with a pre-emptive strategy using other antifungal agents may be the best way of reducing mortality associated with IFI in patients undergoing chemotherapy for acute myeloid leukaemia.

In conclusion, this prospective observational multi-institutional study from India (MISFIC) reports an overall IFI incidence of 26.5% in patients with AML undergoing chemotherapy with an IFI incidence of proven/probable IFI of 8.5%. The use of posaconazole was associated with a lower incidence of IFI. The diagnosis of IFI was not associated with a lower overall survival which is probably related to the better use of antifungal drugs. Newer strategies are needed to reduce mortality associated with IFI.

Acknowledgements This study was funded through an education grant from Merck, Sharpe and Dome (MSD).

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest.

Human and Animal Participants All research involving human participants was performed as per the guidelines established by the Helsinki declaration. All participating institutions obtained ethical clearance from their respective institutions before starting the trial.

Informed Consents All subjects were included only after signing an informed consent.

References

1. Auberger J, Lass-Flörl C, Ulmer H et al (2008) Significant alterations in the epidemiology and treatment outcome of invasive fungal infections in patients with hematological malignancies. *Int J Hematol* 88(5):508–515
2. Caira M, Candoni A, Verga L et al (2015) Pre-chemotherapy risk factors for invasive fungal diseases: prospective analysis of 1,192 patients with newly diagnosed acute myeloid leukemia (SEIFEM 2010—a multicenter study). *Haematologica* 100(2):284–292
3. Gomes MZR, Mulanovich VE, Jiang Y, Lewis RE, Kontoyiannis DP (2014) Incidence density of invasive fungal infections during primary antifungal prophylaxis in newly diagnosed acute myeloid leukemia patients in a tertiary cancer center, 2009 to 2011. *Antimicrob Agents Chemother* 58(2):865–873
4. Girmenia C, Micozzi A, Piciocchi A et al (2014) Invasive fungal diseases during first induction chemotherapy affect complete remission achievement and long-term survival of patients with acute myeloid leukemia. *Leuk Res* 38(4):469–474
5. Michallet M, Bénet T, Sobh M et al (2012) Invasive aspergillosis: an important risk factor on the short- and long-term survival of acute myeloid leukemia (AML) patients. *Eur J Clin Microbiol Infect Dis* 31(6):991–997
6. Tang JL, Kung HC, Lei WC et al (2015) High incidences of invasive fungal infections in acute myeloid leukemia patients receiving induction chemotherapy without systemic antifungal prophylaxis: a prospective observational study in Taiwan. *PLoS ONE* 10(6):e0128410
7. Cho SY, Lee DG, Choi SM et al (2015) Posaconazole for primary antifungal prophylaxis in patients with acute myeloid leukaemia or myelodysplastic syndrome during remission induction chemotherapy: a single-centre retrospective study in Korea and clinical considerations. *Mycoses* 58(9):565–571
8. Korula A, Abraham A, Abubacker FN, Viswabandya A, Lakshmi KM, Abraham OC, Rupali P, Varghese GM, Michael JS, Srivastava A, Mathews V, George B (2017) Invasive fungal infection following chemotherapy for acute myeloid leukaemia—experience from a developing country. *Mycoses* 60(10):686–691
9. Cornely OA, Maertens J, Winston DJ et al (2007) Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. *N Engl J Med* 356(4):348–359
10. Barreto JN, Beach CL, Wolf RC et al (2013) The incidence of invasive fungal infections in neutropenic patients with acute leukemia and myelodysplastic syndromes receiving primary antifungal prophylaxis with voriconazole. *Am J Hematol* 88:283–288
11. Ananda-Rajah MR, Grigg A, Downey MT et al (2012) Comparative clinical effectiveness of prophylactic voriconazole/posaconazole to fluconazole/itraconazole in patients with acute myeloid leukemia/myelodysplastic syndrome undergoing cytotoxic chemotherapy over a 12- year period. *Haematologica* 97(3):459–463
12. Malhotra P, Makkar A, Guru Murthy GS et al (2014) Empirical amphotericin B therapy on day 4 or day 8 of febrile neutropenia. *Mycoses* 57(2):110–115
13. Freifeld AG, Bow EJ, Sepkowitz KA et al (2011) Clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 update by the Infectious Diseases Society of America. *Clin Infect Dis* 52(4):e56–e93
14. Cornely OA, Cuenca-Estrella M, Meis JF et al (2014) European Society of Clinical Microbiology and Infectious Diseases (ESCMID) fungal infection study group (EFISG) and European Confederation of Medical Mycology (ECMM) 2013 joint guidelines on diagnosis and management of rare and emerging fungal diseases. *Clin Microbiol Infect* 20:1–4
15. Tissot F, Agrawal S, Pagano L et al (2017) ECIL-6 guidelines for the treatment of invasive candidiasis, aspergillosis and mucormycosis in leukemia and hematopoietic stem cell transplant patients. *Haematologica* 102(3):433–444

16. Hsu LY, Lee DG, Yeh SP et al (2015) Epidemiology of invasive fungal diseases among patients with haematological disorders in the Asia-Pacific: a prospective observational study. *Clin Microbiol Infect*. <https://doi.org/10.1016/j.cmi.2015.02.019>
17. Jha AK, Bansal D, Chakrabarti A, Shivaprakash MR, Trehan A, Marwaha RK (2013) Serum galactomannan assay for the diagnosis of invasive aspergillosis in children with haematological malignancies. *Mycoses* 56(4):442–448
18. De Pauw B, Walsh TJ, Donnelly JP et al (2008) Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis* 46(12):1813–1821
19. van de Peppel RJ, Visser LG, Dekkers OM, de Boer MGJ (2018) The burden of Invasive Aspergillosis in patients with haematological malignancy: a meta-analysis and systematic review. *J Infect* 76(6):550–562
20. Soyer N, Kiper Ünal HD, Vural F, Şahin F, Töbü M, Dönmez A, Tombuloğlu M, Arda B, Saydam G (2017) Epidemiology and analysis of invasive fungal infections in patients with hematological malignancies: a single-center real-life experience. *Turk J Med Sci* 47(5):1535–1542
21. Tormo M, Pérez-Martínez A, Calabuig M, Hernández-Boluda JC, Amat P, Navarro D, Solano C (2018) Primary prophylaxis of invasive fungal infections with posaconazole or itraconazole in patients with acute myeloid leukaemia or high-risk myelodysplastic syndromes undergoing intensive cytotoxic chemotherapy: a real-world comparison. *Mycoses* 61(3):206–212
22. Dahlén T, Kalin M, Cederlund K, Nordlander A, Björkholm M, Ljungman P, Blennow O (2016) Decreased invasive fungal disease but no impact on overall survival by posaconazole compared to fluconazole prophylaxis: a retrospective cohort study in patients receiving induction therapy for acute myeloid leukaemia/myelodysplastic syndromes. *Eur J Haematol* 96(2):175–180
23. Lee CH, Lin C, Ho CL, Lin JC (2018) Primary fungal prophylaxis in haematological malignancy: a network meta-analysis of randomized controlled trials. *Antimicrob Agents Chemother*. <https://doi.org/10.1128/aac.00355-18>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.