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ORIGINAL ARTICLE

Incidence of bacterial and fungal infections in newly diagnosed acute myeloid leukaemia patients younger than 65 yr treated with induction regimens including fludarabine: retrospective analysis of 224 cases

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Abstract

Objectives: Infections are the major cause of morbidity and mortality in patients with acute myeloid leukaemia (AML). They primarily occur during the first course of induction chemotherapy and may increase the risk of leukaemia relapse, due to a significant delay in consolidation therapy. The intensification of induction chemotherapy and the use of non-conventional drugs such as fludarabine are considered responsible for the increased risk of infections. *Methods:* In this study, we retrospectively analysed the infections occurred in 224 newly diagnosed AML patients ≤65 yr, consecutively treated between 1997 and 2002 with an induction regimen including fludarabine, arabinosyl cytosine and idarubicin, with or without etoposide (FLAI/FLAIE), in the context of three multicentric prospective trials (AML97, AML99, AML02). *Results:* During the induction phase, 146 (65%) patients experienced fever of undetermined origin (FUO), 30 (13%) and 47 (21%) patients had Gram-negative and positive bacteremias, respectively, and 10 (4%) patients developed a probable/proven invasive fungal infection (IFI). The fatality rate for Gram-negative, Gram-positive bacteremias and probable/proven IFI was 10%, 8% and 60% respectively. During consolidation, 75 (35%) patients had FUO, 43 (20%) and 40 (19%) patients had Gram-negative and positive bacteremias, respectively, and 5 (2%) patients developed a probable/proven IFI. The fatality rate for Gram-negative, Gram-positive bacteremias and probable/proven IFI was 14%, 5% and 80% respectively. Interestingly, the overall incidence of microbiologically documented infections during induction was 38% and the incidence of probable/proven IFIs during the induction/consolidation programme was 7%. No infections caused by viruses or opportunistic pathogens were observed neither during induction, nor during consolidation. *Conclusions:* These data, although retrospectively collected, suggest that fludarabine-based chemotherapy is not associated with an increased incidence of infections, in particular IFIs, compared to conventional regimens commonly used for AML induction.

Key words acute leukaemia; induction; fludarabine; fever; infections

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Bacterial and fungal infections are one of the major causes of morbidity and mortality in patients with acute myeloid leukaemia (AML), as a consequence of the use of more and more intensive therapies. The majority of published papers concerning this topic reports that most of infections and almost half of those sustained by fungi occur during the first course of induction chemotherapy (1–9). They may cause not only an increased risk of death, but also an increased risk of leukaemia relapse, because of a significant delay in consolidation and intensification therapy. Therefore, reducing the risk of infections, especially during the induction phase, is a priority, although this objective is apparently hampered by the progressive intensification of anti-leukaemic therapy that has been adopted in the last years.

Addition of a third drug to the standard combination of anthracycline and arabinosyl cytosine (Ara-C) or the use of high-dose Ara-C (HD-AC) are the most common approaches to intensify induction (10–22). We attempted to improve induction's efficacy with a non-conventional induction regimen of fludarabine in combination with intermediate-dose Ara-C and standard dose idarubicin (FLAI) (23–27). The rationale of this combination was that fludarabine is slightly myelotoxic (28–30), it potentiates the anti-leukaemic effects of Ara-C and anthracyclines (31–33), and it is *in vitro* insensitive to P-glycoprotein (p. 170), a drug-transporter protein related to multidrug resistance of leukaemic cells (31, 33). The high efficacy and the low toxicity of FLAI as induction regimen in AML patients <65 yr, was demonstrated first in a pilot study (25) and then in a randomised trial (26). A third prospective non-randomised multicentre study testing a four drugs induction regimen [fludarabine, idarubicin, Ara-C and etoposide (FLAIE)] was started in 2002, but the results have not been published yet.

As fludarabine is a potent immunosuppressive agent, we considered the hypothesis that immunosuppression together with the neutropenia induced by fludarabine-based chemotherapies could increase the risk for infective complications, especially those sustained by fungi. The pro-infective immunosoppressive effect of fludarabine-based chemotherapies was well documented in the setting of patients affected by chronic lymphoprolipherative disorders treated with fludarabine-combined chemotherapies (34–37). In this subset of patients, the T-cell depletion together with the concomitant possible monocyte and B-cell depletion induced by fludarabine, caused a significant increase in infections sustained by viruses and opportunistic pathogens [cytomegalovirus (CMV), herpes simplex virus, *Listeria monocytogenes* and *Pneumocystis carinii*]. No data are available for what concerns infections in AML patients treated with fludarabine-containing regimens, but we think that the expectation of an increased risk of infections has been one of the reasons for the limited use of fludarabine for induction chemotherapy in AML.

In this study, we retrospectively analysed the incidence of infections in 224 newly diagnosed AML patients consecutively treated with a fludarabine-containing induction regimen in three multicentric trials performed in 11 haematological Italian centres between 1997 and 2002 (25–27). The data were then compared with historical data reported for patients treated with standard induction regimens not containing fludarabine (2, 7, 38–41).

Patients and methods

Patients

Between 1997 and 2002, 224 newly diagnosed AML patients <65 yr were enrolled in three consecutive multicentric clinical trials (AML97, AML99 and AML02) testing the efficacy and toxicity of two fludarabine-containing induction regimens, FLAI and FLAIE (25–27). All the enrolled patients were included in this analysis. The trial protocols were approved by local Ethical Committees and the trials were carried out in accordance with the good medical practice principles. Inclusion criteria were: previously untreated AML, age between 18 and 65 yr, performance status (WHO) ≤ 2, absence of co-morbidities that could substantially influence treatment and toxicity (cardiac or renal failure, acute or chronic hepatitis, alcoholism and drug addiction) and written informed consent.

One hundred and thirty-five (60%) patients were induced with FLAI [AML97 trial ($n = 70$) and AML99 trial ($n = 65$)]. Eighty-nine (40%) patients were induced with FLAIE (AML02 clinical trial).

Treatment plans

The treatment programmes for the patients enrolled in the AML97 and AML99 clinical trials have been published elsewhere (25–27). Briefly, in the AML97 clinical trial induction with FLAI for 5 d (fludarabine 25 mg/sqm i.v. infusion days 1–5, Ara-C 2 g/sqm i.v. infusion days 1–5 and idarubicin 10 mg/sqm i.v. infusion on days 1, 3 and 5) was followed by a consolidation with FLAI for 3 d in complete remission (CR) patients or MEC-4 in resistant patients (mitoxantrone 12 mg/sqm i.v. on days 1–4, Ara-C 1 g/sqm on days 1–4 and etoposide 100 mg/sqm on days 1–4). In the AML99 clinical trial, FLAI (5 d) was followed by HD-AC (3 g/sqm/12 h i.v. infusion on days 1–6). The FLAIE induction regimen in the AML02 trial consisted of fludarabine 25 mg/sqm i.v. infusion on days 1–5, Ara-C 2 g/sqm i.v. infusion on days 1–5, idarubicin 6 mg/sqm i.v. infusion on days 1, 3 and 5 and etoposide 100 mg/sqm i.v. infusion on days

1–5. After induction, all the patients underwent consolidation with HD-AC (2 g/sqm i.v. infusion on days 1–5) and idarubicin (12 mg/sqm i.v. infusion on days 1, 3 and 5).

All the patients shared the same strategy for intensification, that was allogenic (allo) or autologous (auto) stem cell transplantation, according to the disease risk at diagnosis, the age and the availability of HLA-compatible sibling or unrelated donor (25–27).

The response evaluation, in terms of CR, non-responders (NRs) and death during induction (DDI) was performed according to the previously published criteria (25–27). Induction death was defined as any death before day +28 from chemotherapy start, with hypocellular marrow (25–27).

Anti-infective policy

All the patients enrolled in the three consecutive trials followed the same policy of prophylaxis and therapy for febrile neutropenia and infections.

Anti-microbial prophylaxis with fluoroquinolone antibiotics (e.g. levofloxacin 500 mg/d) and antifungal drugs (e.g. oral itraconazole 200 mg/b.i.d.) was given during neutropenia until recovery to $>1 \times 10^9$ neutrophils/L. Acyclovir prophylaxis was not routinely administered in this series of patients. Granulocyte colony stimulating factor (G-CSF) was administered only in patients with severe active infections. It was not administered during neutropenia in order to accelerate haematological recovery. Surveillance cultures for bacterial and fungal growth from blood and urine samples and mucosal swabs were performed at the time of hospitalisation and in case of fever (temperature >38°C); standard chest radiographs were obtained at hospital admission and once to twice weekly. CMV reactivation was not routinely tested. In case of clinical suspect of infections, CMV antigenemia was tested.

The diagnostic work-up in case of fever was homogeneous for all the patients included in this analysis. In case of fever (temperature >38°C), after collection of blood cultures from central venous catheter and peripheral vein and collection of nasal, pharyngeal, anal and genital swabs, treatment with broad-spectrum antibiotics (i.e. ceftazidime or piperacillin + tazobactam with or without aminoglycoside) was promptly started. Glycopeptides were added in case of clinically infected catheter entry sites. This empirical therapy was continued until evidence of microbiological isolation, when target therapy was started. In case of resistant fever, defined as the persistence of fever during neutropenia for more than 5 d of broad-spectrum antibiotic treatment in absence of any microbiological evidence, anti-fungal empirical therapy was started. It consisted of amphotericin-B deoxycholate, liposomal amphotericin-B, voriconazole or caspofungin, according to the single centre policy.

Bacterial infections were defined as any confirmed Gram-positive or Gram-negative isolation in the patient's blood or any biological material, including tissue biopsies. Based on clinical, radiological and microbiological data, a diagnosis of possible, probable or proven invasive fungal infections (IFI) was performed, according to the recently published criteria (42, 43). Possible IFIs were diagnosed by the presence of one host factor and one clinical criterion from abnormal site consistent with infection; probable IFIs were diagnosed by the presence of at least one host factor, one microbiological criterion and one clinical criterion. Proven IFIs were diagnosed by histopathological or cytopathological examination showing hyphae or yeast cells from needle aspiration or biopsy specimen with evidence of associated tissue damage; or by positive culture result obtained by sterile procedure from respiratory secretions, sinuses, soft tissue or other organs, excluding urine and mucous membranes for moulds and also sinuses for yeasts. For a better diagnosis of IFI, starting from 2000, a pulmonary high resolution computed tomograpy was performed in case of fever of undetermined origin (FUO) and in the presence of any sign or symptom of pulmonary infection (44, 45). Starting from 2002, basing on the availability of this test in the different institutions, peripheral blood samples for the evaluation of Aspergillus galactomannan EIA were collected at the onset of fever and every 48 h until fever resolution (45). If a radiological sign of infection was observed, bronchoscopy with bronchoalveolar lavage and eventually biopsy was strongly suggested.

Results

Haematological response and toxicity of FLAI/FLAIE

One hundred and fifty-seven out of the 224 AML patients (70%) enrolled in the AML97, AML99 and AML02 clinical trials obtained a CR after a single fludarabine-based induction course; 57 (26%) did not fulfil the criteria for CR and were considered NRs; 10 patients (4%) died during induction (eight infections and two haemorrhages). CR, NRs and DDI rates were comparable in the three protocols (Table 1).

Time to PMN $> 1 \times 10^9$/L for patients treated with FLAI in the AML97 and AML99 clinical trials and with FLAIE in the AML02 clinical trial was 24, 25 and 28 d, respectively (P = NS). Similarly, time to PLTs $> 100 \times 10^9$/L ranged between 23 and 28 d. The only difference of borderline significance was observed between AML97 vs. AML02 ($P = 0.05$) (Table 1).

Grades 3–4 WHO extra-haematological toxicities, including gastro-intestinal (mucositis, diarrhoea, vomiting

Table 1 Clinical characteristics, haematological response and toxicity in 224 newly diagnosed AML patients (pts) consecutively treated with a Fluda-based induction regimen (FLAI/FLAIE)

	AML97 (70 pts)	AML99 (65 pts)	AML02 (89 pts)	*P*-value[2]	Total (224 pts)
Clinical characteristics					
M/F	36/34	37/28	45/44	NS	118/106
Median age (range)	48 (19–64)	47 (19–60)	45 (18–63)	NS	46 (18–64)
Response to induction (%)					
CR	47/70 (67)	47/65 (72)	63/89 (71)	NS	157/224 (70)
NR	20/70 (29)	16/65 (25)	21/89 (24)	NS	57/224 (26)
DDI	3/70 (4)	2/65 (3)	5/89 (5)	NS	10/224 (4)
No of pts who proceeded to first consolidation	67/70 (96)	63/65 (97)	84/89 (94)	NS	214/224 (95)
Haematological toxicity after induction					
PMN > 1×10^9/L	24 (19–45)	25 (20–46)	28 (19–60)	NS[3]	26 (19–60)
Median days (range)					
PLTs > 100×10^9/L	23 (19–40)	25 (19–33)	28 (20–72)	NS[4]	28 (19–72)
Median days (range)					
Extra-haematological toxicity after induction[1]					
Grades III–IV WHO toxicities (Episodes)	6	5	10	NS	21
Rate of infectious deaths (induction)					14/101 (14%)

CR, complete remission; NR, non-responder; DDI, death during induction.
[1]Mucositis, diarrhoea, vomiting, nausea, hyperbilirubinemia, acute renal failure and skin rash.
[2]Chi-squared test.
[3]AML97 vs. AML02: $P = 0.06$.
[4]AML97 vs. AML02: $P = 0.05$–AML99 vs. AML02: $P = 0.12$.

and nausea), hepatic (hyperbilirubinemia) and renal (acute renal failure) toxicities and skin rash, were few and comparable in the patients enrolled in the AML97, AML99 and AML02 clinical trials (6, 5 and 10 episodes, respectively; P = NS) (Table 1).

Infections during the Fluda-based induction

The incidence of FUO, bacterial and fungal infections during induction with FLAI/FLAIE is reported in Table 2. One hundred and forty-six out of 224 patients (65%) developed FUO, 30 (13%) and 47 patients (21%) experienced a Gram-negative or Gram-positive bacteremias respectively. The incidence of Gram-negative bacteremias in CR and resistant patients was 16% and 7% respectively, and the most frequent pathogens were Enterobacteriaceae (67%), *Pseudomonas aeruginosa* (27%). The incidence of Gram-negative bacteremias with pneumonia was 13% (all observed in patients who entered CR after induction). One out of 30 Gram-negative bacteremias (3%) was associated with soft tissue localisation (in one resistant patients). The incidence of Gram-positive bacteremias in CR and resistant patients was 22% and 21% respectively, and the most frequent pathogens were coagulase-negative staphylococci (59%), *Staphylococcus aureus* (13%), *Streptococcus pneumoniae* (11%) and Enterococcus (11%). The incidence of Gram-positive bacteremias with pneumonia was 6% (all observed in CR patients). Two out of 47 Gram-positive bacteremias (4%) was associated with soft tissue localisation (in two resistant patients). The fatality rate for Gram-negative and positive bacteremias was 10% and 8% respectively. No infections caused by viruses or opportunistic pathogens were observed. Concerning IFIs, 14 out of 224 (6%) patients showed a possible IFI, whereas 10 (4%) cases were identified as probable/proven IFIs, with an overall fatality rate for probable/proven IFIs of 60%. The incidence of possible IFIs in CR and resistant patients was 7% and 5% respectively. The incidence of probable/proven IFIs in CR and resistant patients was 3% and 9% respectively. In particular, proven IFIs were caused by *Aspergillus* sp. (four episodes with pulmonary localisation), *A. fumigatus* (one episode with pulmonary localisation), *A. flavus* (one episode with pulmonary localisation), *Candida glabrata* (one episode with positive haemoculture both from central venous catheter and peripheral blood) and *C. krusei* (one episode of candidal sepsis). Three of these patients entered CR, four were resistant and one died during induction. The IFI resolved in all the three patients who entered CR after induction [two pulmonary aspergillosis (*Aspergillus* sp. and *A. flavus*) and one candidal sepsis (*C. krusei*)]. In contrast, the IFIs did not resolve in all the four resistant patients [four pulmonary aspergillosis (one *A. fumigatus* and three *Aspergillus* sp.)]. A *C. glabrata* sepsis was responsible for DDI in one patient.

As reported in Table 2, the incidence of fever and infections in the three consecutive clinical trials was

Table 2 Incidence of FUO, bacterial and fungal infections in 224 newly diagnosed AML patients (pts) consecutively treated with a fludarabine-based induction regimen (FLAI/FLAIE)

	AML97 (70 pts)	AML99 (65 pts)	AML02 (89 pts)	*P*-value[1]	Total (224 pts)	Fatality rate
FUO (%)	36/70 (51)	39/65 (60)	71/89 (80)	0.0006	146/224 (65)	–
Gram-negative bacteremias (%)	12/70 (17)	9/65 (14)	9/89 (10)	NS	30/224 (13)	3/30 (10)
Enterobacteriaceae (%)	7	7	6		20/30 (67)	
Pseudomonas aeruginosa (%)	3	2	3		8/30 (27)	
Others (%)	2	0	0		2/30 (6)	
With pneumonias (%)	2/12 (17)	1/9 (11)	1/9 (11)		4/30 (13)	
With soft tissue localisations (%)	1/12 (8)	0/9 (0)	0/9 (0)		1/30 (3)	
Gram-positive bacteremias (%)	13/70 (19)	10/65 (15)	24/89 (27)	NS	47/224 (21)	4/47 (8)
Coagulase-negative staphylococci (%)	7	6	15		28/47 (59)	
Staphylococcus aureus (%)	4	0	2		6/47 (13)	
Streptococcus pneumoniae (%)	2	1	2		5/47 (11)	
Enterococcus (%)	0	3	2		5/47 (11)	
Others (%)	0	0	3		3/47 (6)	
With pneumonias (%)	1/13 (8)	1/10 (10)	1/24 (4)		3/47 (6)	
With soft tissue localisations (%)	1/13 (8)	0/10 (0)	1/24 (4)		2/47 (4)	
Possible IFIs (%)	1/70 (1)	5/65 (8)	8/89 (9)	NS	14/224 (6)	1/14 (7)
Probable/proven IFIs (%)	2/70 (3)	3/65 (5)	5/89 (6)	NS	10/224 (4)	6/10 (60)
Proven IFI (%)						
Aspergillus sp.	0	2	2	NS	4/10 (40)	
Aspergillus fumigatus	1	0	0		1/10 (10)	
Aspergillus flavus	1	0	0		1/10 (10)	
Candida glabrata	0	0	1		1/10 (10)	
Candida krusei	0	1	0		1/10 (10)	14/101 (14)

AML, acute myeloid leukaemia; FUO, fever of undetermined origin; IFI, invasive fungal infection; NS, non-significant.
[1]Chi-squared test.

comparable, except for FUO ($P = 0.0006$), that was significantly higher in the AML02 clinical trial than in the other trials. Similar results were obtained when comparing the incidence of FUO for patients enrolled in AML97 and AML99 vs. AML02 clinical trial (data not shown).

Infections during the first consolidation

Two hundred and fourteen out of 224 (95%) patients consecutively treated with a fludarabine-based induction regimen proceeded to first consolidation, after a median time from induction start of 35 d (range 28–52). Consolidation consisted of FLAI-3 or MEC-4 for 47 and 20 patients, respectively (AML97); HD-AC for 63 patients (AML99); and Ida-HD-AC for 84 patients (AML02). The incidence of FUO, Gram-negative and positive bacteremias was 35% (75/214 patients), 20% (43/214 patients) and 19% (40/214 patients) respectively. For what concerns Gram-negative bacteremias, the most frequent pathogens were Enterobacteriaceae (63%), *P. aeruginosa* (28%) and their association with pneumonia was observed in the 9% of cases. Three out of 43 Gram-negative bacteremias (7%) were associated with soft tissue localisation. For what concerns Gram-positive bacteremias, the most frequent pathogens were coagulase-negative staphylococci (75%), Enterococcus (12%) and *S. pneumoniae* (7%) and their association with pneumonia was observed in the 5% of cases. One out of 40 Gram-positive bacteremias was associated with soft tissue localisation (3%). The fatality rate for Gram-negative and positive bacteremias was 14% and 5% respectively. With respect to induction, a statistically significant difference was observed only for the incidence of FUO (65% vs. 35%, $P < 0.0001$; Table 3). Similarly to what observed during induction, no infections sustained by viruses or opportunistic pathogens were observed. Six out of 214 patients (3%) experienced a possible IFI during first consolidation. The incidence of probable/proven IFIs was 2% (five of 214 patients) and the fatality rate for probable/proven IFIs was 80%. Five episodes of proven IFI were recorded, which were *A. fumigatus* (three episodes with pulmonary localisation), *Fusarium* spp. (one episode with blood isolation) and *C. albicans* (one episode with blood isolation and concomitant hepato-splenic localisation). These latter patients were not the same patients who developed a proven IFI during induction. IFI resolved in one out of the three patients who were in CR at the time of consolidation (sepsis with hepato-splenic localisation sustained by *C. albicans*), but it did not resolve in the other two [two pulmonary aspergillosis (*A. fumigatus*)]. The two resistant patients died of

Table 3 Comparison of incidence of FUO and infections in 224 newly diagnosed AML patients (pts) after fludarabine-based induction (FLAI/-FLAIE) and after first consolidation

	Induction (224 pts)	First consolidation (214 pts)	*P*-value[1]	Fatality rate for infections Induction	Consolidation
FUO (%)	146/224 (65)	75/214 (35)	<0.0001	–	–
Gram-negative bacteremias (%)	30/224 (13)	43/214 (20)	NS	3/30 (10)	6/43 (14)
Enterobacteriaceae (%)	20/30 (67)	27/43 (63)			
Pseudomonas aeruginosa (%)	8/30 (27)	12/43 (28)			
Others (%)	2/30 (6)	4/43 (9)			
With pneumonias (%)	4/30 (13)	4/43 (9)			
With soft tissue localisations (%)	1/30 (3)	3/43 (7)			
Gram-positive bacteremias (%)	47/224 (21)	40/214 (19)	NS	4/47 (8)	2/40 (5)
Coagulase-negative staphylococci (%)	28/47 (59)	30/40 (75)			
Staphylococcus aureus (%)	6/47 (13)	0/40 (0)			
Streptococcus pneumoniae (%)	5/47 (11)	3/40 (7)			
Enterococcus (%)	5/47 (11)	5/40 (12)			
Others (%)	3/47 (6)	2/40 (5)			
With pneumonias (%)	3/47 (6)	2/40 (5)			
With soft tissue localisations (%)	2/47 (4)	1/40 (3)			
Possibile IFIs (%)	14/224 (6)	6/214 (3)	NS	1/14 (7)	0/6 (0)
Probable/proven IFIs (%)	10/224 (4)	5/214 (2)	NS	6/10 (60)	4/5 (80)
Proven IFI					
Aspergillus sp. (%)	4/10 (40)	0/5 (0)			
Aspergillus fumigatus (%)	1/10 (10)	3/5 (60)			
Aspergillus flavus (%)	1/10 (10)	0/5 (0)			
Candida glabrata (%)	1/10 (10)	0/5 (0)			
Candida krusei (%)	1/10 (10)	0/5 (0)			
C. albicans (%)	–	1/5 (20)			
Fusarium spp	–	1/5 (20)			

AML, acute myeloid leukaemia; FUO, fever of undetermined origin; IFI, invasive fungal infection; NS, non-significant.
[1]Chi-squared test.

progressive disease and active infection [one pulmonary aspergillosis (*A. fumigatus*) and one sepsis sustained by *Fusarium* spp.].

When considering separately the four patient groups that received consolidation therapy, the only significant statistical difference was the incidence of FUO when FLAI was used as consolidation with respect to AC-based chemotherapy (21% vs. 39%; $P = 0.02$, data not shown). Interestingly, the overall incidence of IFIs in patients treated with FLAI in the consolidation phase was lower than the one observed in patients treated with AC-based consolidation regimens (0% vs. 7%; P = NS; data not shown).

Discussion

The aim of this study was to retrospectively evaluate the effect of fludarabine on the incidence of infective complications when administered in combination with Ara-C and one anthracycline as induction treatment for newly diagnosed AML patients. We analysed the incidence of infections in 224 AML patients consecutively treated from 1997 to 2002 in 11 Italian haematological centres with a fludarabine-based induction chemotherapy (FLAI/FLAIE). As reported in Table 2, the overall incidence of microbiologically documented infections was 38% and the only statistically significant difference was the lower incidence of FUO in patients enrolled in AML97 and AML99 vs. AML02 clinical trials ($P = 0.0006$). This latter aspect is probably related to the observed higher haematological and non-haematological toxicity for patients treated with FLAIE, that is a four drugs induction regimen. Interestingly, no infections caused by viruses or opportunistic pathogens were observed.

Comparing these data with the published ones, we focused on some historical studies which explored the infective toxicity of standard induction regimens for AML patients, with or without the addition of G-CSF (38–41). In these studies, the incidence of microbiologically documented infections after induction varied between 36% and more than 70% for AML patients treated without G-CSF. The median age of our patients was 46 yr, the median time to PMN (neutrophils) recovery ranged between 24 (patients treated with FLAI) and 28 d (patients treated with FLAIE) and all the patients received antimicrobial prophylaxis with oral quinolone and itraconazole. According to these data, our reported

incidence of microbiologically documented infections is comparable with the 36% reported by Heil *et al.* (Table 4), even though the median age of Heil's series is 8-yr older than our.

Concerning fungal infections, we observed an incidence of probable/proven IFIs after induction and consolidation of 7% (15 episodes in 224 patients). One of the largest studies on fungal infections in haematological malignancies recently published by Pagano *et al.* (7) reports a 12% of incidence of IFIs in AML patients over a similar period of time (Table 4). While we focused on induction and first consolidation, Pagano *et al.* analysed the incidence of IFIs during the whole treatment programme of AML. This could explain the different incidence of IFIs in the two studies. However, taken together, these data suggest that fludarabine in combination with intermediate dose of Ara-C and standard dose of anthracycline, with or without etoposide, does not increase the risk of fungal infections. Among the probable factors that may be responsible for these favourable data, the very low haematological and extra-haematological toxicities (especially for what concerns mucositis) observed with FLAI/FLAIE play the major role. This latter aspect may be related to the use of intermediate dose of Ara-C over 2-h infusion, instead of higher doses or continuous infusion. The higher haematological toxicity of FLAIE (AML02) compared with FLAI (AML97 and AML99) appeared to increase the incidence of FUO but did not influence the incidence of microbiologically documented infections.

To investigate if the immunosuppressive effect of fludarabine may become evident later, we also evaluated the incidence of infections during consolidation regimens. With respect to induction, a statistically significant difference was observed only for FUO ($P = 0.0001$; Table 2). On the other hand, a trend towards a higher incidence of Gram-negative bacteremias after consolidation was observed. This may be partially related to the impact of the consolidation employed in patients enrolled in AML02 (idarubicin and HD-AC), that may have increased the incidence of mucositis, in some cases. Moreover, the low incidence of IFIs observed in the 47 patients treated with FLAI as consolidation may be partially related to the shorter duration of neutropenia when FLAI was used [median time to ANC $> 0.5 \times 10^9$/L for FLAI: 12 d (range 10–20) vs. 22 d (range 15–36) for non-fludarabine-based regimens].

Some limitations of this study should be mentioned. The data are retrospectively collected and the analysed patients were treated over a relatively long period of time. This latter aspect is of interest, because prophylaxis, diagnosis and treatment of infections, particularly of IFIs, have significantly changed during the last 10 yr (6–8). In particular, many new technologies [galactomannan and beta-glucan antigens as well as high-resolution computed tomography (44, 45)] have become available for the diagnosis of IFIs, in order to better characterise the group of possible and probable infections. Unfortunately, some of these biological and radiological data are lacking for some of our patients and the application of

Table 4 Incidence of infections in AML patients

References	No of pts	Age	Induction	Microbiological documented infections	Fungal infections
(41)	26 AML	Median: 45 yr (26–59)	DNR + Ara-C	73%[1]	–
(39)	262 AML	Median: 54 yr (16–88)	DNR + Ara-C + VP-16	36%[1]	–
(40)	126 AML	Mean: 66.5 yr (55–75)	Ida + Ara-C	72%[1] Septicemia: 28% Pulmonary infections: 28% Other documented infections: 33%	11%[2]
(38)	105 AML	Median: 67 yr (56–84)	DNR + Ara-C	78%[1]	15%[3]
(2)	673 IA[4]	Median: 40.3 yr (<1–89)	–	–	8%[4]
(7)	3012 AML	>16 yr	DNR/Ida/Mito + Ara-C +/– VP-16	–	12%[5]
Our series	224 AML	Median: 46 yr (18–64)	FLAI/FLAIE	38%[1] Bacteremias: 34% Bacteremias with pneumonia: 9% Bacteremias with soft tissue localisations: 4%	7%[5]

AML, acute myeloid leukaemia; DNR, daunorubicin; Ara-C, cytarabine; VP-16, etoposide; Ida, idarubicin; Mito, mitoxantrone; IFI, invasive fungal infections; IA, invasive aspergillosis.

[1]Data refer to induction.

[2]The reference reports on serious fungal infections.

[3]The reference reports on IFI.

[4]The reference reports on the epidemiology of IA in France between 1994 and 1999 in AML.

[5]Data on probable/proven IFIs refer to induction and consolidation.

the most recent diagnostic criteria for IFIs (42, 43) may be considered hazardous for our series. Nevertheless, the reported incidence of FUO and infections after a Fluda-based induction has been confirmed in three consecutive multicentric trials and this may contribute to abolish the bias of a single-centre effect. Notably, this is particularly true for the incidence of probable/proven IFIs after induction and consolidation therapy (7%), which favourably compares with the one reported by Cornet *et al.* (8%) (2) and by Pagano *et al.* (12%) (7) (Table 4).

We can finally conclude that fludarabine-based induction chemotherapy is not associated with an increased incidence of infections, in particular IFIs, compared to conventional regimens commonly used for AML induction.

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