Proceeding Paper

Prevalence and Genotyping of *Pneumocystis jirovecii* Pneumonia in Patients with Previously Untreated Acute Myeloid Leukemia [†]

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Abstract: *Pneumocystis jirovecii* pneumonia (PJP) is an opportunistic infection affecting immunocompromised patients. Patients with acute myeloid leukemia (AML) are not considered at high risk of PJP, thus, prophylaxis is not recommended. Between 2010 and 2020 we retrospectively analyzed 251 AML patients. We performed molecular diagnosis and genotyping of *Pneumocystis jirovecii* in 67 bronchoalveolar lavage samples. Eleven cases of PJP were diagnosed, with a prevalence of 4.3%. Our study confirms that the most widespread genotype in Europe is genotype 1; in our patients, 70% presented with genotype 1 and 30% the genotype 3.

Keywords: *Pneumocystis jirovecii* pneumonia; acute myeloid leukemia; bronchoalveolar lavage; genotyping

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1. Introduction

Pneumocystis jirovecii (PJ) is an ascomycetous fungus that causes opportunistic infections in humans. PJ pneumonia (PJP) is a life-threatening interstitial pneumonia that mostly occurs in immunocompromised patients such as those with acquired immunodeficiency syndrome (AIDS) and lymphoproliferative disorders, as well as allogeneic hematopoietic stem cell transplantation (HSCT) recipients [1–3]. Since 2005, the European Conference on Infections in Leukemia (ECIL) has aimed to produce recommendations for the management of infections in patients with hematologic malignancies (HM) [4]. The ECIL recommend prophylaxis with daily or three-times weekly trimethoprim/sulfamethoxazole (TMP-SMX) in patients with strong evidence of high risk of PJP. The current guidelines do not extend to acute myeloid leukemia (AML); although PJP has been observed in patients with AML not receiving HSCT, data on incidence are sparse, and mostly based on case reports [5]. Generally, those patients are not considered at high risk of PJP development, due to the unusual administration of high dose of steroids during induction therapy [6]. This retrospective study aimed to evaluate the incidence of PJP during AML-induction therapy, and to evaluate the need for prophylaxis. Additionally, we performed molecular diagnosis and genotyping of PJ in bronchoalveolar lavage (BAL) samples [7].

2. Materials and Methods

We retrospectively analyzed 251 consecutive newly diagnosed adult (≥ 18 years old) patients with non-promyelocytic AML, admitted between January 2010 and May 2020 to

the Hematology Ward of the “Policlinico Universitario di Roma Tor Vergata” to receive induction therapy. High-resolution computed tomography (HRCT) of the chest was routinely performed in all patients as part of their initial evaluation before the start of AML-induction therapy, in cases of fever persisting for more than 72 hours following antibiotic therapy initiation or with the presence of respiratory signs/chest pains not otherwise explained [8]. Sixty-seven BALs were performed in patients with pneumonia of unclear etiology or non-response to antibiotics [9]. BAL samples were evaluated at the parasitology laboratory, using the mtLSU-rRNA gene as a diagnostic marker for the detection of *P. jirovecii* and the determination of the specific genotype of the species [10]. Molecular analysis was carried out by PCR amplification and subsequent sequencing to highlight single nucleotide polymorphisms (SNP) in the sequence. Amplified DNA was sequenced by Bio-Fab Research and multiple alignments were performed using Clustal W2 and MEGA7 software to find a correlation between the genotype and the patient's characteristics. The mtLSU-rRNA locus distinguished 4 genotypes on the basis of polymorphisms in positions 85 and 248: genotype 1 (85:C/248:C), genotype 2 (85:A/248:C), genotype 3 (85:T/248:C) and genotype 4 (85:C/248:T) [11]. All patients have consented, through informed consent, to the processing of their sensitive data.

3. Results

During the study period, 251 new diagnoses of non-promyelocytic AML were made (153 males and 98 females, median age 62); of these, 172 were submitted to intensive chemotherapy, 43 to hypomethylating agents or low dose cytarabine, and 36 were found unfit to receive active AML treatment and received only supportive therapy. None of the patients received steroids for more than 15 days, and the maximum dosage used was 20 mg prednisone/day. None of the patients had received previous TMP/SMX prophylaxis. Sixty-seven BALs were performed (Table 1). PJ presence was demonstrated in 11/67 (16.7%) of BALs with a prevalence of 4.3% (11/251, 11 male), median age 71 (range 63–84 years). In our patients, 8/11 (73%) presented with genotype 1 and 3/11 (27%) with genotype 3. No statistically significant correlations have yet been found between these patients.

Table 1. Patient characteristics.

N. of Patients	251
Sex	
Male	153 (61%)
Female	98 (39%)
Age	Median: 62 yrs
<60 yrs	145 (57.8%)
>60 yrs	106 (42.2%)
BAL performed:	67
Prior to first-line therapy	39 (58.2%)
After first-line therapy	28 (43.3%)
AML Treatment	
Intensive chemotherapy	179 (71.3%)
Non-intensive chemotherapy	36 (14.3%)
Supportive care	36 (14.3%)
PJ + BAL	11/67 (16.4%)
Prior to first-line therapy	2/39 (5.1%)
After first-line therapy	9/28 (32%)
Comorbidities	
None	105 (41.8%)
One or more	146 (58.2%)

Table 1. Cont.

N. of Patients	251
Co-present lung infections	
Aspergillosis	5/11 (45.5%)
Bacterial pneumonia	1/11 (9%)
CMV	1/11 (9%)
None	3/11 (27.3%)
Lung HRTC scan findings	
Typical	6/11 (54.5%)
Atypical	5/11 (45.5%)

BAL, bronchoalveolar lavage; CMV, cytomegalovirus; HRTC, High-resolution computed tomography; PJ, *Pneumocystis jirovecii*.

4. Conclusions

PJP is an opportunistic fungal infection commonly found in HM with wide variability based on the type of malignancy and treatment. In our experience, the incidence of PJP during AML induction therapy was not negligible (4.3% among the whole series of patients and 16.7% of pneumonias of unknown origin or unresponsive to antibiotics). Accordingly, our study showed that surveillance is important and awareness of PJP in AML patients is necessary. Prophylaxis for PJP, which results in mortality benefits when the estimated risk of infection exceeds 3.5%, as previously reported, should be considered [12]. From a molecular point of view, our study confirms that the most widespread genotype in Europe is genotype 1 [10]. Further prospective studies are needed to assess the actual indications for prophylaxis, to evaluate PJP incidence during the subsequent course of AML and to assess the exact timing for prophylaxis initiation and duration.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The authors declare no conflict of interest.

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