MORTALITY RATE AND PROGNOSTIC FACTORS FOR POOR OUTCOME IN HIV-INFECTED BULGARIAN PATIENTS WITH PNEUMOCYSTIS PNEUMONIA OVER A 3-YEAR PERIOD

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ABSTRACT:

Background: In spite of the use of combination antiretroviral therapy and specific prophylaxis, Pneumocystis pneumonia remains one of the most common AIDS-defining diseases with high mortality rate. The aim of this study was to analyze the cases of pneumocystis pneumonia over a three-year period by means of assessing the mortality rate and the prognostic factors for the outcome of the disease.

Material and methods: Epidemiological, clinical, laboratory and demographic data were analyzed for 13 HIV-infected patients with proved pneumocystis pneumonia, hospitalized at the AIDS Department over the period January 2017 - December 2019. For data processing different methods of descriptive statistics were used.

Results: All presented patients had extremely severe immune deficiency and other opportunistic infections or AIDS-related diseases. The diagnosis of pneumocystis pneumonia was confirmed with PCR in 76.9% of the patients and with Giemsa staining for cysts and trophozoites in 23.1% of the patients. Most of our patients were male (84.6%) and the leading mechanism of HIV infection was unprotected homosexual contact. The mean age of the patients presented was 37.4 ± 10.27. Our results showed high PJP mortality (46.3 %), despite the applied etiological therapy.

Conclusions: *Pneumocystis jirovecii* pneumonia (PJP) continues to be a life-threatening infection in HIV-infected patients with severe immune suppression. PJP mortality remains high, and is significantly dependent on age, male sex, low CD4 T cell count, high HIV viral load, low blood albumin, and the number and severity of comorbidities.

Keywords: *Pneumocystis jirovecii pneumonia, prognostic factors, mortality*

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INTRODUCTION:

Pneumocystis pneumonia remains the most common AIDS-defining disease in the United States, although its mortality rate has dropped from over 50 to 10% (1, 2, 3). The causative agent is *Pneumocystis jirovecii*, which was initially classified as a type of trypanosome, and subsequently, as a protozoa. In 1988, its DNA was found to be that of an atypical fungal species (4).

The use of combination antiretroviral therapy and prophylaxis for pneumocystosis has resulted in a dramatic reduction in the number of cases in the US and in Western Europe. However, a significant increase in the cases of pneumocystis pneumonia is observed in certaine regions such as sub-Saharan Africa, where *Pneumocystis jirovecii* was previously a rare pathogen (5).

Until 1980, it was a rare disease, affecting mainly malnourished children with severe immunodeficiency and adults with severe immunosuppression, mainly related to chemotherapy for neoplastic diseases. With the onset of the HIV pandemic, the spread of pneumocystis pneumonia increased dramatically, and it is becoming the most common AIDS-defining disease in the developed countries (5, 6).

Following the introduction of co-trimoxazole chemoprophylaxis in HIV-infected patients with CD4+ T lymphocyte counts below 200 cells/mm^3 since 1989, and especially after the introduction of highly-active antiretroviral therapy (HAART) in the mid-1990s, there has been a significant decline in the disease in developed countries in Europe its prevalence has

been reduced from 4.9 cases per 100000(1994) to 0.3 cases per 100 000 (1999), (7, 8).

This is a retrospective study of patients with pneumocystis pneumonia (PJP), hospitalized and treated at the AIDS Department over a three-year period. We analyzed the patients' demographic, epidemiological, and clinical characteristics. We also analyzed the impact of some factors, such as gender, age, comorbidities, and some laboratory test values on the disease course and outcome.

MATERIAL AND METHODS:

We present data of 13 HIV-infected patients with proven pneumocystis pneumonia. They were hospitalized at the AIDS Department of the Specialized Hospital for Active Treatment of Infectious and Parasitic Diseases in Sofia over the period 2017–2019. Routine clinical, laboratory and microbiological tests were carried out in the respective laboratories of the Specialized Hospital for Active Treatment of Infectious and Parasitic Diseases in Sofia. The immunological and virological studies regarding the HIV infection were performed at the National Center of Infectious and Parasitic Diseases.

The diagnosis of pneumocystis pneumonia in ten of the patients was confirmed by means of PCR methods: Real-time PCR with TaqMan probes for detection of *Pneumocystis jirovecii*. In the other three patients, diagnosis was confirmed by means of morphological methods: Giemsa staining for cysts and trophozoites**.**

Statistical analysis was performed using descriptive statistics methods (descriptives, cross tabulations, frequencies); analysis of statistical significance was carried out by means of parametric (one sample T – test) and non-parametric methods (one sample Kolmogorov – Smirnov test). The level of rejection of null hypothesis was set at $p < 0.05$.

RESULTS:

Table 1. Patients' demographic and epidemiological characteristics

Patient №	Sex	Age	Year of HIV diagnosis	Year of PJP diagnosis	Route of HIV transmission
1	Female	35	2017	2017	HETERO
2	Male	32	2014	2018	HETERO
3	Male	45	2019	2019	MSM
4	Male	44	2014	2019	MSM
5	Male	32	2019	2019	MSM
6	Male	39	2019	2019	HETERO
7	Female	27	2019	2019	HETERO
8	Male	42	2018	2019	MSM
9	Male	53	2019	2019	HETERO
10	Male	14	2009	2019	MCT
11	Male	45	2019	2019	MSM
12	Male	32	2016	2019	MSM
13	Male	47	2019	2019	MSM

Thirteen patients were monitored: 11 male (84.6%) and 2 female (15.4%), aged 37.4 ± 10.27 over the interval 14–53 years (Table 1).

The mechanism of HIV infection was as follows: heterosexual in 5 patients (38.5%), homosexual in 7 patients (53.8%), and mother-to-child transmission in 1 patient (7.7%).

The diagnosis of PJP related to the time of the diagnosis of the HIV infection was done as follows:

- In the same year: in 8 patients (61.5%);
- 2 years later: in 1 patient (7.7%);
- 3 years later: in 1 patient (7.7%);
- 4 years later: in 1 patient (7.7%);
- 5 years later: in 1 patient (7.7%);
- 10 years later: in 1 patient (7.7%).

The patients diagnosed with pneumocystis pneumonia in the year of detection of their HIV infection (8 of the patients) were late presenters, and pulmonary symptoms were the reason to be examined and detect their HIV infection. The other five patients were aware of their HIV infection, but had not been registered nor followed up at the Department of AIDS until the manifestation of their pulmonary symptoms. The patients' comorbidities are presented in Table 2.

Table 2. Patients' comorbidities

Patients' comorbidities	Number of patients	Percentage
Cryptosporidiosis	1	7.7
Candidiasis, disseminated	3	23
Orofaryngeal candidiasis	5	38.5
H. influenzae tonsillitis	1	7.7
Cyrrhosis; Aethilismus	1	7.7
Pulmonary tuberculosis	1	7.7
Kaposi sarcoma	1	7.7

Blood count, biochemical parameters and acid-base balance were examined in all patients. The variations in the values of these laboratory parameters are presented in Table 3.

Table 3. Variations in the values of some laboratory parameters

Basic lab tests	Number of patients	Minimum	Maximum	Mean	Std. Deviation
Hemoglobin	13	98.00	147.00	122.8462	15.76307
Leucocytes	13	2.40	11.10	7.0000	2.58618
Platelets	13	128.00	625.00	275.5385	130.26743
Albumin	13	15.00	35.00	22.7077	15.26458
ALAT	13	15.00	71.00	28.8462	20.15708
pO_2	13	24.20	80.00	42.6923	15.60627

The main indicators of HIV infection were as follows: CD4+ T cells: 20 ± 26 cells/mm^3 in the range 2–85 cells/mm^3; mean HIV viral load (VL): 496,673 ± 452,056 copies/µl in the range 29,821–1,455,994 copies/µl.

The diagnosis of pneumocystis pneumonia was confirmed as follows:

- PCR: in 10 patients (76.9%);
- Giemsa staining for cysts and trophozoites: in 3 patients (23.1%).

All patients were treated with TMP/SMX for PJP at a dose of 120 mg/kg intravenously. For two of them, Clindamycin was added at a dose of 3 x 600 mg intravenously.

In eleven patients antiretroviral therapy was initiated, the other two died before initiating it.

The administered antiretroviral regimens are presented in Table 4.

Table 4. Antiretroviral regimens

ART regimen	Number of patients	Percentage
ABC/3TC + DRV/r	1	7.7
ABC/3TC/DTG	6	46.2
FTC/TDF+ DTG	1	7.7
FTC/TDF + RAL	2	15.4
3TC+TDF+LPV/r	1	7.7

In 6 patients lethal outcome was observed, with two of them having the most severe concomitant diseases: Kaposi sarcoma with visceral localization, and cirrhosis. In five of the deceased patients, the immediate cause of death was acute respiratory failure. The cirrhotic patient died of cirrhosis complications: a hemorrhagic syndrome.

The lethal outcome was significantly associated with the following indicators at a significance level for rejection of the null hypothesis $p < 0.05$:

- gender ($p < 0.0011$);
- CD4 T < 20 cells /mm^3 ($p < 0.05$);
- HIV VL > 300,000 copies/ml ($p < 0.01$);
- number and severity of comorbidities ($p< 0.0011$);
- $pO_2 < 40$ mmHg ($p < 0.0011$);
- albumin < 30 g/l ($p< 0.011$)

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DISCUSSION

Our AIDS center monitors about 1,200 people living with HIV (PLHIV), which accounts for 2/3 of all PLHIV, followed up in Bulgaria. 97% of the patients who are followed-up in our ward receive antiretroviral therapy. This retrospective study covered a small number of HIV-positive patients with pneumocystis pneumonia. All of them had severe immune deficiency and had taken neither antiretroviral therapy, nor PJP prophylaxis before their pneumocystis pneumonia was detected. This small number of patients indicates that pneumocystis pneumonia is indeed rare among PLHIV in the era of combination ART and PJP prophylaxis (5, 7, 8).

Patients were diagnosed with the help of either PCR, or staining methods, which are the established diagnostic methods for this condition (9, 10, 11, 12, 13, 14).

We treated patients with TMP/SMX intravenously at the dose 120 mg/kg/d, and continued in accordance with the recommendations for the treatment of pneumocystis pneumonia (15). In two of the patients with rapid progression of the disease, we added Clindamycin. As Pentamidine and Caspofungin are not available in Bulgaria, This decision was based on literature data, showing a good response to Clindamycin in patients with pneumocystis pneumonia (16). Unfortunately, in both patients the disease ended with a lethal outcome.

The PJP mortality rate is high among patients not previously treated with antiretroviral therapy and with delayed diagnosis and treatment (17, 18, 19). In our study the overall mortality was high: 46.15%. All of our patients were late presenters that had not previously received ART or prophylaxis.

We observed a significant association of the poor prognosis of the disease with male sex, age over 40 years, albumin below 30 g/l, the number and severity of comorbidities, pO2 < 40 mmHg, CD4 T cell count below 20 cells/mm^3, and HIV VL over 500,000 copies/µl.

In line with our results other studies have also indicated that age > 35 years, $PaO_2 < 8$ kPa and serum albumin level < 30 g/l at admission were poor prognostic factors (20, 21, 22). Another study found that the presence of Kaposi's sarcoma as a concomitant disease was a poor prognostic factor (23), which was also confirmed in our study.

CONCLUSIONS

Pneumocystis pneumonia (PCP) is a potentially life-threatening infection that occurs in HIV-infected patients with severe immune suppression. Despite the general belief that PJP is uncommon in the era of the universal guidelines for immediate initiation of combination ART, this disease is still a problem in our country due to the still late diagnosis of the HIV infection in some patients. PJP mortality continues to be high, and significantly depends on the age, male sex, low CD 4 T+ cell count, high HIV VL, low

blood albumin, and the number and severity of comorbidities.

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