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RESEARCH ARTICLE

# c.202G > A/c.376A > G G6PD Polymorphisms Increase the Risk of Fungal Infections in Acute Myeloid Leukemia Patients

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## ABSTRACT

***Introduction:*** **Patients with acute myeloid leukemia (AML) show a higher risk for several types of infections, including fungal infections (FI), which are one of the main causes of morbidity and mortality. Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme located in all cells that is very necessary in leukocytes for the production of basic and acid proteases that are used to destroy invading microorganisms. Our objective in this study was to evaluate whether polymorphisms in the G6PD gene concomitantly with FI are associated with clinical events and morbidity in patients diagnosed with AML and followed up at the Amazonas State Blood Center (HEMOAM), Manaus, Brazil.**

***Materials and Methods:*** **The study population was randomly constituted of adults and children, of either sex, and any age, with a diagnosis of acute myeloid leukemia, all of whom were undergoing treatment at the HEMOAM. Molecular genotyping was performed using real-time PCR (qPCR) and subsequent Sanger sequencing to confirm the c.202G > A/c.376A > G polymorphisms.**

***Results:*** **A total of 157 patients (91 (58%) males and 66 (42%) females) were involved in the study. The most prevalent AML subtype in the studied group was M3 in 63 patients (40.12%), followed by M5 in 33 patients (21.02%), M2 in 21 patients (13.37%) and M4 in 15 patients (9.55%), with a similar prevalence between genders. The prevalence of fungal infections was identical between genders; however, bruising ($p = 0.004$), vomiting ($p = 0.016$) and cardiac alterations ($p < 0.001$) were higher in females, while persistent cough ($p = 0.049$) and diarrhea ($p < 0.001$) were higher in males. A total of eighteen patients presents G6PD polymorphisms, with 8 (5.1%) of these for $c.202^{GA/AA}$, 18 (11.5%) for $c.376^{AG/GG}$ and 4 (2.5%) for both polymorphisms concomitantly ($c.202^{AA}/c.376^{GG}$). However, the prevalence of death in patients affected with FI was much higher in those that have these polymorphisms ($p < 0.001$).**

***Conclusion:*** **We believe that the determination of G6PD polymorphisms will allow the development of monitoring strategies, and aid in early diagnosis and the appropriate and targeted treatment for AML. In addition, evaluating their activity may help to identify AML patients at a higher risk of FI, thus allowing the design of more intensive therapeutic and surveillance strategies.**

**Keywords:** Amazonas, Fungal Infections, Leukemia, Manaus.

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## 1. Introduction

Leukemias are a set of diseases that are characterized by malignant alterations in the hematopoietic system and are classified as either chronic or acute. Acute myeloid leukemia (AML) occurs due to clonal proliferation of myeloblasts that accumulate in the bone marrow and inhibit normal hematopoietic activity and are characterized by anomalous proliferation of immature bone marrow precursors [1]–[4]. It occurs mostly in adults over 60 years old and is more frequent in males. In Brazil, in the three years between 2020–2022, there were approximately 6000 cases of leukemia, representing a crude rate of 5.67 cases per 100,000 inhabitants, with a death rate of 60% [5]. AML patients undergoing chemotherapy are at increased risk of infections, including fungal infections, which are the main causes of morbidity and mortality [6], [7].

Some current studies have shown an increase in fungal infections in AML patients with concomitant glucose-6-phosphate dehydrogenase (G6PD) deficiency [8]–[11]. The G6PD enzyme is located in all cells. Still, it has the primordial function in erythrocytes of protecting against oxidative stress, preventing their early hemolysis, and also in leukocytes for the production of their basic and acid proteases contained in their granules for the destruction of invading microorganisms [12].

G6PD deficiency is a common genetic disorder that affects approximately 400 million people worldwide, which is equivalent to approximately 5.0% of the population [13]. Currently, studies have described approximately 200 clinically relevant mutations of G6PD deficiency. The most prevalent variants reported with enzymatic deficient worldwide include G6PD "A" (c.376A > G); G6DP "A-" (c.202G > A, c.376A > G) and G6PD Mediterranean (c.563C > T). The c.563C > T variant has decreased enzyme activity (<10%), leading to severe hemolytic crises, while c.202G > A and c.376A > G variants, have ranged from 10% to 60%, with carriers usually asymptomatic, however, they may have moderate or severe hemolytic crises medication-induced [14], [15].

This study aimed to determine the genetic polymorphisms in the G6PD gene in patients diagnosed with AML and followed up at the Amazonas State Blood Center (HEMOAM), and compare them with the presence of fungal infections.

## 2. Materials and Methods

### 2.1. Study Design

The population of this study was randomly constituted of a total of 157 patients, among them adults and children, of either sex and any age, with a diagnosis of acute myeloid leukemia, all of whom were undergoing treatment at the Amazonas State Blood Center (HEMOAM) during the period from February 2009 to October 2019. All patients were recruited by spontaneous demand, always after consultation with a hematologist. All of them were instructed about the project and invited to participate in the research, when they accepted, they signed the informed consent form. In the case of minors, a term of assent was signed by their legal guardian.

Following the inclusion criteria (diagnosis of acute myeloid leukemia) and after the agreement of each patient, the participants were invited to answer a sociodemographic and clinical questionnaire. Of the total of 157 patients who were included in the study and who agreed to participate, 107 are yet currently being followed up at the HEMOAM Foundation and 50 have since died. A survey of physical and electronic medical records, such as the I-Doctor and Soft-lab programs implemented at the HEMOAM, was also carried out. All data were collected with the consent of each patient and with the consent of the Foundation. The information obtained was kept confidential and this study was carried out under strict scientific and ethical principles.

The main information collected from these medical records was regarding the current history of the disease, microbiological test results, chest X-rays, signs of infections with febrile neutropenia, alterations in the oral mucosa, length of treatment, AML subtype and initial and final blood count with values of blasts when supplied.

All blood samples were collected and cultured using BacT/ALERT 3D BacT/ALERT® 3D 60 Blood Culturing System (Biomerieux Brasil). When positive, each isolate was subcultured on sabouraud dextrose agar to ensure its viability, purity, and to susceptibility testing. The isolated pathogens were identified and subjected to antimicrobial susceptibility testing using an automated VITEK® 2 Compact system (Biomerieux Brasil). The same was done for all urine samples, seeded directly on sabouraud dextrose agar with later identified in the VITEK 2 according to the manufacturer's instructions.

For some laboratory analyses, such as hemogram and total leukocyte count, blood samples of between 2 to 5 ml were collected in 127 patients via venipuncture in a tube containing EDTA. In contrast, in another 30 samples, only DNA was extracted using peripheral blood or bone marrow slides stored in the patients' respective medical records, since they had died before the study started. These 30 samples are part of a biorepository for the storage and use of biological material stored in the form of DNA, serum, or plasma in specific −80 °C freezers and used only for genetic molecular testing purposes, which were previously authorized by the research participant or their guardian in research projects for use of up to 10 years of storage. Genomic DNA was extracted using a HiYield™ Genomic DNA extraction kit (BioAmerica Inc., USA). After extraction, the DNA was quantified using a spectrophotometer (NanoDrop™ 2000, Thermo Fisher Scientific, Massachusetts, EUA), and then stored at −20 °C.

### 2.2. G6PD Genotyping

Genotyping of the SNPs was conducted using TaqMan® SNP genotyping assays (Applied Biosystems, Foster City, CA) on a real-time PCR system (QuantStudio 6 and 7 Flex, Applied Biosystems). The amplification reaction was

TABLE IA: Probes of Real-time PCR Assays using TaqMan Technology to Detect the G6PD Polymorphisms

| Coding DNA position [A] | Nucleotide variation (Ref/Alt) | Ref SNP identifier [B] | Minor allele frequency [C] |
|---|---|---|---|
| 202 | G > A | rs1050828 | A = 0.03 |
| 376 | A > G | rs1050829 | G = 0.07 |
| 542 | A > T | rs5030872 | T = 0.01 |
| 563 | C > T | rs5030868 | T = 0.03 |
| 968 | T > C | rs76723693 | C = 0.02 |
| 1033 | A > G | rs5030869 | G = 0.01 |
| 1339 | G > A | rs137852317 | A = 0.01 |

Note: A: Originally described cDNA positions in different manuscripts focused on G6PD polymorphisms. B: RefSNP accession ID (rs number): https://www.ncbi.nlm.nih.gov/snp/. C: Brazil minor allele frequency: https://www.ncbi.nlm.nih.gov.

TABLE IB: Synthetic Oligonucleotides to DNA Sequencing used to Amplify exons 4 and 5 of the G6PD Gene [16]

| Mutation | Forward primer (5′-3′) | Reverse primer (5′-3′) | Exon | Fragment size (pb) |
|---|---|---|---|---|
| c.202G > A | TTACAGCTGTGCCCTGCCCT | AGGGCAACGGCAAGCCTTAC | IV | 919 |
| c.376A > G | CTGCGTTTTCTCCGCCAATC | AGGGCAACGGCAAGCCTTAC | IV | 585 |

Note: F: forward; R: reverse; bp: B*ase Pairs*.

performed for a final volume of 10 uL/reaction, which contained 4 uL of 2× TaqMan Universal Master Mix, 0.3 uL of 20× SNP Genotyping Assay, 3.8 uL of sterile water, and 2.0 uL of DNA (~100 ng) of the sample. The probes used are shown in Table IA. To confirm the polymorphisms found, amplification of the relevant DNA segments using PCR, followed by DNA sequencing (ABI 3100, Applied Biosystems, Foster City, CA) was performed (Table IB).

### 2.3. *Statistical Analysis*

Demographic and clinical characteristics were summarized as the median and range or number. Incidence of FI was the primary endpoint, while the secondary endpoints were the impact of the presence of G6PD polymorphisms presence on the incidence of fungal infections, Candida sepsis, overall survival, and infection-related death. Significant differences were calculated using Fisher's two-sided exact test or Pearson's chi-squared test, as appropriate. Categorical variables were detected using the chi-square test to compare the differences between the two groups. Overall, survival was analyzed using the Kaplan–Meier method, which was calculated from the date of diagnosis of leukemia with only fungal infection to death or the last follow-up time. Data were entered into a database using Graphpad Prism (version 5.0 Graphpad Software, San Diego, CA, USA) and IBM SPSS Statistics (version 19, IBM Corp., Armonk, NY, USA). Values of $<0.05$ were considered statistically significant.

## 3. Results

### 3.1. *Sociodemographic Profile*

Of the total of 157 patients, 91 (58%) were male and 66 (42%) were female. Of these, 149 (94.90%) were born and reside in the Brazilian Amazon, with 133 (84.7%) belonging to the state of Amazonas, while 5 (3.25%) were born in Rondônia and 3 (1.91%) in Roraima, though, they have lived for at least 10 years in the state of Amazonas. The average age among men was 38.08 ± 24.01 years, while the average age in women was 40.86 ± 23.14 years. Most patients declared themselves to be brown-skinned. Regarding the level of education, it was observed that men had a lower level of education and, in both genders, less than 10% had higher education (Table II).

### 3.2. *AML Subtypes*

The most prevalent AML subtype in the studied group was M3, which was found in 63 patients (40.12%), followed by M5 in 33 patients (21.02%), M2 in 21 patients (13.37%), and M4 in 15 patients (9.55%), with a similar prevalence between genders. In addition to these, 5 cases of unspecified biphenotypic leukemias were diagnosed with a frequency of 3.18% (5) (Table III).

### 3.3. *Clinical Events*

The information contained in the socio-epidemiological questionnaire and medical records showed fever as the most common event in both genders (53.8% vs 54.5%), which was followed by anemia (6.5% vs 16.7%), and arm and leg pain (13.2% vs 21.2%), for males and females, respectively. The prevalence of fungal infections was identical between genders; however, bruising ($p = 0.004$), vomiting ($p = 0.016$), and cardiac alterations ($p < 0.001$) were higher in females, while persistent cough ($p = 0.049$) and diarrhea ($p < 0.001$) were higher in males (Table IV). Other less frequent events were observed, such as systemic arterial hypertension, gastritis, and cardiac alterations, with 2.2%, 2.3%, and 7.6%, respectively (data not shown).

In our study, we found episodes of recurrent infections in females to be more frequent than in males, such as urinary tract infection, intestinal infection, and otitis, with frequencies of 6.9% vs 1.1%, 5.5% vs 1.1% and 2.7% vs 1.1% in females and males, respectively; however, no statistical significance was observed. Males, on the other hand, presented a higher frequency for furuncles, pneumonia, throat infection, sepsis, sinusitis, skin infection, and hepatitis, 5.5% vs 1.5%, 3.3% vs 0%, 12.1% vs 4.5%, 2.2% vs 0%, 8.8% vs 4.5% and 3.3% vs 1.5% in male and female, respectively. Fungal mucositis, which is quite common in patients undergoing treatment for hematological diseases, presented a similar frequency in both genders, with 12.1% in males and 15.2% in females.

TABLE II: Socio-demographic Characteristics of Patients with a Diagnosis of Acute Myeloid Leukemia at HEMOAM

| Patient Data | | Male | Female | Total |
|---|---|---|---|---|
| | | 91 (58%) | 66 (42%) | 157 (100%) |
| **Age (means ± SD)** | | 38.08 ± 24.01 | 40.86 ± 23.14 | 39.25 ± 23.64 |
| **Race** | Brown | 84 (92.31) | 57 (86.2) | 141 (89.81) |
| | White | 7 (7.69) | 9 (13.8) | 16 (10.19) |
| **Schooling** | IE | 48 (52.75) | 22 (33.34) | 70 (44.59) |
| | CE | 19 (20.88) | 10 (15.16) | 29 (18.47) |
| | IM | 6 (6.59) | 6 (9.09) | 12 (7.64) |
| | CM | 6 (6.59) | 19 (28.78) | 25 (15.92) |
| | IHE | 4 (4.40) | 4 (6.06) | 8 (5.10) |
| | IHE | 8 (8.79) | 5 (7.57) | 13 (8.28) |

Note: IE: Incomplete Elementary School; CE: Complete Elementary; IM: Incomplete Middle school; CM: Complete High School; IHE: Incomplete Higher Education; CHE: Complete Higher Education.

TABLE III: French-American-British (FAB) Classification of Acute Myeloid Leukemia Subtypes and Numbers of Samples at HEMOAM

| Population | | Male | Female | Total |
|---|---|---|---|---|
| | | n (%) | n (%) | n (%) |
| **Subtypes** | M0 | 1 (1.09) | 1 (1.51) | 2 (1.27) |
| | M1 | 4 (4.39) | 3 (4.54) | 7 (4.45) |
| | M2 | 14 (15.38) | 7 (10.60) | 21 (13.37) |
| | M3 | 38 (41.75) | 25 (37.87) | 63 (40.12) |
| | M4 | 9 (9.89) | 6 (9.09) | 15 (9.55) |
| | M5 | 18 (19.78) | 15 (22.72) | 33 (21.02) |
| | M6 | 2 (2.19) | 0 (0) | 2 (1.27) |
| | M7 | 2 (2.19) | 7 (10.60) | 9 (5.73) |
| | Biphenotypic | 3 (3.29) | 2 (3.03) | 5 (3.18) |
| **Total** | | 91 | 66 | 157 (100.0) |

Note: M0: Undifferentiated acute myeloblastic; M1: Acute myeloblastic leukemia with minimal maturation; M2: Acute myeloblastic leukemia with maturation; M3: Acute promyelocytic leukemia; M4: Acute myelomonocytic leukemia; M5: Acute monocytic leukemia; M6: Acute erythroid leukemia; M7: Acute megakaryoblastic leukemia.

TABLE IV: Most Frequent Clinical Events Reported by Acute Myeloid Leukemia Patients at HEMOAM

| Clinical event | | Male | Female | RP | CI (95%) | P value |
|---|---|---|---|---|---|---|
| Fever | No | 42 | 30 | 1.01 | 0.76–1.29 | 0.936** |
| | Yes | 49 | 36 | | | |
| Anemia | No | 76 | 55 | 0.98 | 0.69–1.43 | 0.975** |
| | Yes | 15 | 11 | | | |
| Pancytopenia | No | 86 | 58 | 0.45 | 0.13–1.35 | 0.136** |
| | Yes | 05 | 8 | | | |
| Bruises | No | 81 | 47 | 0.38 | 0.32–0.92 | 0.004** |
| | Yes | 10 | 19 | | | |
| Cough | No | 83 | 65 | 5.87 | 1.21–2.08 | 0.049* |
| | Yes | 8 | 1 | | | |
| Vomiting | No | 88 | 57 | 0.24 | 0.06–0.83 | 0.016* |
| | Yes | 3 | 9 | | | |
| Pain (arms and legs) | No | 79 | 52 | 0.65 | 0.49–1.19 | 0.181** |
| | Yes | 12 | 14 | | | |
| Diarrhea | No | 87 | 66 | 7.69 | 1.53–2.02 | <0.001* |
| | Yes | 7 | 0 | | | |
| Cardiac alterations | No | 91 | 61 | 7.57 | 0.52–0.68 | <0.001* |
| | Yes | 0 | 05 | | | |

Note: RP: Prevalence Ratio; CI: Confidence interval. *Fisher exact probability test. *$\chi^2$ test (Yates's corrections).

Several infectious agents were reported in the medical records. Among them, *Candida tropicalis, Malassezia sp, Candica sp, Rothia dentocariosa,* Streptococcus. *mitis,* Klebsiella. *oxytoca, Staphylococcus hyicus, Stenotrophomonas maltophilia, Acinetobacter iwoffii, Rhodotorula mulaginosa, Cryptococcus laurentii,* Serratia

TABLE V: Demographics Characteristics in AML Patients Positive for the $c202^{AA}$ and $c.376^{GG}$ Polymorphisms of G6PD

| N | Gender/Age | Ethnicity | c.202G > A | c.376A > G | AML subtype | Fungal infection |
|---|---|---|---|---|---|---|
| 1 | F/8 | Brown | GG | AG | M7 | No |
| 2 | F/43 | Brown | GG | AG | M3 | Skin |
| 3 | M/24 | White | AA* | GG* | M3 | Legs |
| 4 | F/45 | Brown | GG | AG | M3 | No |
| 5 | M/18 | Brown | GG | GG* | M3 | Mucositis |
| 6 | M/83 | Brown | AA* | GG* | M0 | No |
| 7 | M/46 | Brown | AA* | AA | M2 | Mucositis |
| 8 | F/8 | Brown | GG | AG | M5 | No |
| 9 | F/41 | Brown | GG | AG | M3 | Mucositis |
| 10 | M/2 | Brown | AA* | GG* | M2 | Throat |
| 11 | F/39 | Brown | GA | AG | M1 | Skin |
| 12 | F/64 | Brown | GG | AG | M0 | No |
| 13 | F/38 | Brown | GA | AG | M3 | No |
| 14 | M/67 | Brown | GG | GG* | M1 | Mucositis |
| 15 | F/50 | Brown | GG | AG | M3 | Mucositis |
| 16 | F/12 | White | GG | AG | M3 | No |
| 17 | F/69 | Brown | GG | AG | M2 | No |
| 18 | M/07 | Brown | AA* | GG* | M2 | Mucositis |

Note: M: Male; F: Female; N: Patient Numbers. 202G > A: GG: Normal Homozygous Allele; GA: Heterozygous Allele; AA: Mutant Homozygous Allele. c.376A > G: AA: Normal Homozygous Allele; AG: Heteroygous Allele; GG: Mutant Homozygous Allele. Chatham (c.1003G > A), Santiago de Cuba (c.1339G > A) and A-(c968T > C) variants were not found in or study. *Hemizygous Mutant (Only male).

TABLE VI: Genotypic Frequency Distribution of G6PD Polymorphisms among Fungal Infection in AML Patients

| AML Patients | | c.202 N (%) IC | | p-value RR | c.376 N (%) IC | | p-value RR | c.202/376 N (%) | | p-value RR IC |
|---|---|---|---|---|---|---|---|---|---|---|
| | | AA | GG | | GG | AA | | AA/GG | GG/AA | |
| Fungal Infection | Yes | 4 (9,8) | 37 (92) | 0.114** 2.83 0.72–12.71 | 5 (12,2) | 36 (87,8) | 0.864* 1.09 0.35–3.04 | 3 (7,3) | 38 (92,7) | 0.055** 8.49 0.81–221.3 |
| | No | 4 (3,4) | 112 (96,6) | | 13 (11,5) | 103 (88,8) | | 1 (0,9) | 115 (99,1) | |

Note: N: cases. * $\chi^2$ test (Yates's corrected). **Fisher's exact test. c.202G > A: GG: Normal Homozygous Allele; GA: Heterozygous Allele; AA: Mutant Homozygous Allele. c.376A > G: AA: Normal Homozygous Allele; AG: Heterozygous Allele; GG: Mutant Homozygous Allele.

*marcescens were diagnosed, as well as Staphylococcus saprophyticus, Staphylococcus hugdunesis, Pseudomonas aerogenosa, Staphylococcus epidermidis, Staphylococcus aureus, Acinetobacter baumanni, Staphylococcus haemolyticus, Staphylococcus intermedius, K*lebsiella *pneumoniae, Staphylococcus pneumoniae, Staphylococcus saprophyticus* and herpes zoster.

Interestingly, almost all the detected infectious agents were reported as mono-infections, except for *S. pneumoniae*, which has always been reported in conjunction with *S. epidermidis*.

### 3.4. *G6PD Genotypes*

In the study, only $c.202^{GA/AA}$ and $c.376^{AG/GG}$ G6PD polymorphisms were found, with 8 patients (5.1%) for $c.202^{GA/AA}$, 18 (11.5%) for $c.376^{AG/GG}$ and 4 (2.5%) for both polymorphisms concomitantly ($c.202^{AA}/c.376^{GG}$). Other G6PD polymorphisms analyzed, such as the Mediterranean (c.563C > T), Santamaria (c.542A > T), Chatham (c.1003G > A), Santiago de Cuba (c.1339G > A), and A-(c968T > C) variants were not found (Table V). Cross-table correlations between fungal infections and G6PD genotypes c.202 and c.376 in patients with acute leukemia are shown in Table VI. However, the results for the homozygous genotype mutant c.202AA and the double homozygous mutant 202/376AA/GG showed interesting values, RR: 2.83 and RR:8.49, respectively.

The mutated genotypes of G6PD were more frequent in women with brown skin and the M3 subtype, with mucositis and throat infection being the most reported infections. Kaplan–Meier estimates of Fungal-free survival during follow-up showed a clear increase in risk for patients with G6PD polymorphisms (Figs. 1A–1C), and the prevalence of death in patients with the polymorphisms found was much higher when affected by FI ($p < 0.001$).

The $c.202^{GA/AA}$ variant was more frequent in women, while $c.376^{AG/GG}$ was more frequent in men; though there was no statistical significance between genders when associated with infections. Although a more significant sample size is necessary for possible association, we were able to detect a prevalence of 12.1% of G6PD polymorphisms in patients with AML, with the highest frequency in the M3 subtype and with random infections. We found that the prevalence of death in patients with polymorphisms was much higher when affected by fungal infections.

## 4. Discussion

The main clinics in G6PD-deficient individuals are described with jaundice and drug or infection-induced hemolytic anemia and normally are mostly due to point mutations [17], however, too implicated in many diseases and pathophysiological processes, including inflammation, microbial infection, and sepsis [18]. G6PD-deficient

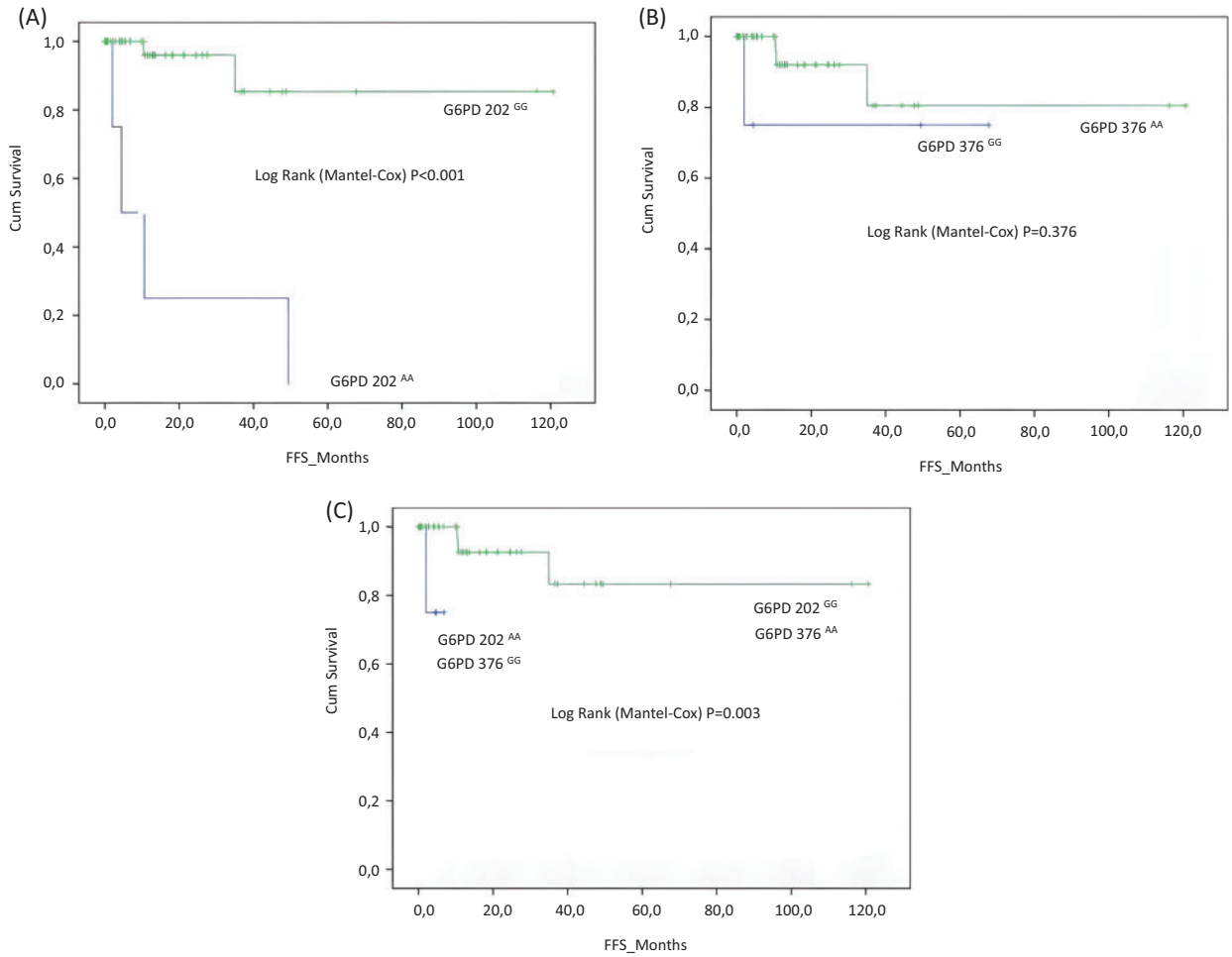


Fig. 1. Fungal-free survival (FFS) in acute myeloid leukemia patients in two groups of G6PD polymorphisms (c.202G > A / c.376A > G): **(A)** Patients with wild-type homozygous genotype ($c.202^{GG}$) versus patients with homozygous mutation ($c.202^{AA}$); **(B)** Patients with wild-type homozygous genotype ($c.376^{AA}$) versus patients with homozygote mutation ($c.376^{GG}$); **(C)** Patients with wild-type homozygotes genotypes ($c.202^{GG}/c.376^{AA}$) versus patients with homozygous mutations ($c.202^{AA}/c.376^{GG}$).

individuals have more events from pathogen infections owing principally to their decreased ability to activate the innate immune response and in part, to decrease inflammasome activation and the bactericidal response [19]. In addition, some virus infections, such as influenza virus, HIV, and influenza A, in G6PD-deficient subjects, cause an increase in oxidative stress to accelerate its replication [20].

We believe that due to the small number of patients positive for G6PD mutations, the cross-correlations between fungal infections and the c.202G > A/c.376A > G G6PD genotypes did not demonstrate statistical significance. We recognize that in a study with a larger number of patients, cross-correlations may respond to this theory. Therefore, we consider that the results of this study corroborate studies that support the hypothesis that mutations that lead to G6PD deficiency may confer an increased risk of fungal infection in AML patients [21]–[22].

Several studies have identified risk factors for the development of mainly fungal infections in hematological neoplasms, among them: immunosuppressed patients undergoing induction or consolidation chemotherapy with mucositis, prolonged neutropenia, and underwent organ transplantation [23]. Around the world, fungal diseases affect over a billion people, killing about 1.5 million each year. Patients with hematological diseases are part of a group in which serious fungal infections occur, mainly aspergillosis and candidiasis [24].

Recently, an epidemiological survey at HEMOAM, between the years 2017 to 2018, detected fungal infections in 25 patients, and of these, 16% were patients with AML [25]. Sanna *et al*. demonstrated a high incidence of fungal infections in patients with AML, including invasive infections, and found, significantly, almost 7 times more infections in AML patients concomitantly with the G6PD enzyme deficiency [10]. These same authors emphasized in this same study the fundamental role of G6PD in the response to infections and proved that patients with G6PD deficiency had their granulocytic activity of neutrophils reduced by less than 30%. We believe that this result occurred due to G6PD, in addition to being a key enzyme in the pentose-phosphate pathway and supply (of ADPH against oxidative stress), also promotes responses to the oxidative explosion of neutrophils against aggressive microorganisms such as fungi [10].

Gafter-Gvilli observed that bacterial infections had a 9-times higher frequency in those patients with AML concomitantly with G6PD deficiency when compared to those with AML and normal activity for G6PD. In addition, these authors also demonstrated a longer hospital stay for

patients with G6PD deficiency compared to non-deficient patients [11]. It is important to note that in the studies mentioned above, only the activity of the enzyme G6PD was analyzed. In these studies, the minimum value of 10% of activity was considered for the patient to be considered deficient in the enzyme. It should be noted that molecular studies were not carried out to determine the polymorphism and its variants. In addition, the acute myeloid leukemia subtypes were not stratified.

Unfortunately, in our study, it was not possible to define the species of infectious agent that could be directly correlated to the carriers of the G6PD polymorphisms, since not all of them had a specific infection during treatment. However, patients with the mutated genotypes of G6PD 202/376 (data not shown) at some point had prolonged febrile neutropenia and at least one episode of infection, including fungal infection accompanied by mucositis.

Although our study did not determine G6PD enzyme activity, the genotypic variants found could predict its activity and associate it with clinical events in the patient. This study reported a high frequency of African variants c.202G > A/c.376A > G in AML patients in the state of Amazonas among individuals in the region who were brown-skinned, corroborating the higher prevalence of these variants in most of Brazil [26]–[29]. Added to these data, the results of this study corroborate those found in the literature, which demonstrates frequencies close to what was observed and that these are linked to a higher occurrence in patients with AML, mainly during treatment [9].

## 5. Conclusion

In Brazil, our study is a pioneer in describing acute myeloid leukemia patients and their subtypes with genotypic variants of the G6PD enzyme and possible fungal infections. It was observed that when AML patients have G6PD polymorphisms this can influence the risk of developing FIs, in particular, G6PD polymorphisms that have enzyme deficiency of lower than 60% (such as c.202$^{AA}$ found in this study), which seems to significantly increase fungal infections in these patients.

We believe that the determination of G6PD polymorphisms can allow the development of monitoring strategies, and the appropriate and targeted treatment in acute myeloid leukemia patients. In addition, evaluating their G6PD activity and/or polymorphisms may help to identify AML patients at a higher risk of FIs, thus allowing the design of more intensive therapeutic and surveillance strategies.

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## Ethics Statement

This project was approved by the Ethics and Research Committee (CEP) of the HEMOAM, and developed according to the criteria of the Regulation of Bioethics in Brazil, Resolution 466/2012 of the National Health Council. All participants or guardians (in the case of patients under 18 years of age) signed the written consent form and the study was approved by CEP under protocol number 3700618 CAAE 83413718600000009.

## Author Contributions

All authors contributed to critically editing the manuscript for intellectual content and provided approval of its final version.

## Conflicts of Interest

Authors declare that there are no conflicts of interest and that they have no financial relationships with the industry.

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