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Case Report

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Moleculargenetic and in Addition Partly Discrepant Infection Serological Malaria Testing in Two Blood Donors

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Malaria · Malaria PCR testing · Malaria infectious serology

Abstract
Introduction: According to the guidelines (GL) valid in Germany, persons born or raised in a malaria-endemic area or had continuously stayed in a malaria-endemic area for more than 6 months may only be admitted donating blood if, among other things, validated and quality-assured laboratory diagnostics show that there is no evidence of infectivity. In a statement of the Working Group "Blood" of the Federal Ministry of Health (WGB), a reduction of the deferral period from 4 to 3 years and an antibody test after the deferral period are recommended. ***Methods:*** In accordance with the GL, nucleic acid testing (NAT) by means of PCR is carried out at our institution after a retention period of 4 years. In addition to the validated molecular biological testing, an infection serological examination was performed. ***Case Presentation:*** In the present cases, *Plasmodia* genome was detected in the respective single PCR in two blood donors originating from malaria-endemic areas after the expiry of the deferral period. However, one donor tested negative for antibodies against *Plasmodia*. ***Discussion/Conclusion:*** This observation is discussed in the context of a recommendation of the WGB. The question is addressed whether PCR testing is dispensable or whether a combination of infection serological testing and NAT should be favored.

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Introduction

Plasmodia as the causative agent of various forms of malaria can be transmitted by transfusion of blood and blood products [1]. Donors at increased risk of transmitting latent malaria infections include people born or raised in a malaria-endemic area [2]. According to the current German guidelines, people who are at risk must be deferred from blood donation for 4 years after leaving the endemic region and may only be admitted to donation thereafter if a targeted medical history, clinical examination, and validated and quality-assured laboratory diagnostics give no hint for infectiousness [2]. The discussion about the significance of transfusion-related malaria has recently increased in Germany as immigration from malaria-endemic areas to Germany and Europe is likely to increase [3]. At the DRK Blutspendedienst West gGmbH (BSD), testing is performed by means of nucleic acid amplification analysis in the form of individual PCR. The prerequisite for this is that the donor provides truthful information during the specific medical history and medical examination in combination with the blood donation. In a statement from February 2022, the Working Group on Blood recommends a reduction of the deferral period to 3 years and antibody testing after the deferral period [3]. This recommendation is considered critical in view of the cases described. This case report would like to contribute to the current discussion in Germany

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and the world about the importance and the most effective strategies to avoid transfusion-related malaria infections [3].

Case Report

Testing on the donor examination is performed routinely using NAT (PCR): sample extraction and pipetting of the PCR plate is performed using the AltoStar AM 16 system from altona Diagnostics, sample pretreatment: 500 µL AltoStar Whole Blood Pretreatment Buffer 1.5 + 500 µL EDTA-anticoagulated whole blood, sample input volume: 535 µL stabilized, liquefied whole blood per preparation with AltoStar Purification Kit 1.5, purification of the *Plasmodia* DNA in 65 µL eluate, of which 10 µL is used in the PCR with the RealStar Malaria PCR Kit 1.0. The manufacturer specifies 1.27 IU/µL eluate (95% confidence interval 0.57–5.42 IU/µL) as the detection limit with 95% hit probability. According to the probit analysis, the laboratory-internal validation with International Reference Material IS #04/176 of the WHO resulted in a 95% LOD of 501 IU/mL sample (conf. interval 301–1,023 IU/mL sample). PCR is then performed in the Bio Rad LightCycler (CF×96 Real-Time PCR Detection System).

In addition to the validated molecular biological testing, an infection serological examination was performed. An ETI-Max ELISA system from DiaSorin was used with an Anti-*Plasmodia* IgG assay from Euroimmun according to the operating instructions: the test contains microtiter strips of 8 reagent wells each coated with a mixture of appropriate recombinant target antigens of all human pathogenic *Plasmodial* species. The reagent tubes are incubated with diluted patient plasma samples in the first analysis step. In the case of positive samples, spread IgG antibodies bind to the respective antigens. To visualize these antibodies, the next step involves incubation with an enzyme-labeled anti-human IgG, which catalyzes a subsequent color reaction. Unfortunately, this test is not approved for the examination of blood donors in Germany.

At National Reference Centre, the Bernhard Nocht Institute for Tropical Medicine (BNITM), the identical PCR assay is applied for confirmation testing. No residual sample volume from the donors was left for comparison testing with another PCR-based assay.

Donor A is a 20-year-old man who was born and raised in Nigeria. He immigrated to Germany in 2017. The PCR was performed according to the manufacturer’s instructions. The PCR result was clearly positive with cycle threshold values (Cq) of 24.60 and 24.56, respectively, and an ELISA OD of 2.839. Our result was confirmed by the BNITM using EDTA blood or plasma and the same PCR test as our laboratory. The BNITM malaria PCR was “positive”; in the malaria PCR differentiation, *Plasmodium malariae* was detected. Infectious serology testing (IIFT) performed by the BNITTM was also positive.

Donor B is a 35-year-old man who was born and raised in Syria. After fleeing Syria via Iraq, he immigrated to Germany 6 years ago. In the PCR performed as for donor A, the threshold cycle was slightly but reproducibly exceeded with a Cq value of 44.01 and 43.40, respectively, so that the analysis resulted “positive.” The malaria PCR performed for the control role at BNITM was also positive. Malaria PCR differentiation was negative due to low nucleic acid concentration in the sample. Infectious serology testing, performed as in donor A, remained negative with an OD value of 0.086.

Both donors were clinically without pathological findings at the time of donation and were certified as suitable for donation by the physician. No blood products were released from the two donors.

Discussion/Conclusion

The observed cases show, in agreement with the courses described in literature, that malaria can still be latently active years after infection [1, 3]. In the cases presented, the donors had been outside endemic areas for 5 and 6 years, respectively. Furthermore, it should be noted that both donors were considered medically suitable to donate. Despite a high pathogen load, donor A also appeared clinically healthy. Unfortunately, tracking of both donors was not possible because their residential addresses had changed after donor examination, and their current locations are unknown to the blood transfusion service. In both cases, molecular biological detection of the pathogen genome was successful. The detectability of antibodies against *Plasmodia* was only successful in one of the 2 cases, which also corresponds to the data in the literature, in which a sensitivity of infection serological methods of between 53 and 64% is assumed [3]. Donor B would also have been presumed to be infectious. Both cases should trigger further reflection on the deferral period and diagnostics in donors from malaria-endemic areas. Both cases encourage our blood transfusion service to continue with molecular biological testing for the *Plasmodial* genome [4, 5] and to consider adding infection serological screening as part of a study. The development of methods for pathogen and antibody detection that are as sensitive as possible should be pursued further [3, 6]. Malaria infectivity is quite high [3]. As few as 10 parasites are presumed the infectious dose of transfusion-transmitted malaria [3]. Up to 450,000 parasites may be contained in one RBC [7]. 18S rRNA is present in multiple copies per parasite [8] and performs as a sensitive target for all Plasmodium species. RealStar Malaria PCR Kit 1.0 targets 18S rRNA DNA, which is also used by other PCR malaria assays [4]. To our knowledge, no correlation factor between IU and parasites exists, even a correlation between IU and nucleic acid copies has not been established, mainly because of methodological and procedural varieties of NAT-based assays (e.g., sample input volume, extraction method, target design). Exactly, this insufficiency has led to establishing an International Reference Standard (NIBSC #04/176) for NAT by WHO to make harmonization and comparability of NAT-based results, either qualitative or quantitative, possible [9]. Thus, the sensitivity of currently available NAT methods is still not able to meet the demand for detecting the minimum infectious dose for transmission of malaria infection

reported in the literature. Thus, neither NAT nor infectious serology testing is satisfactory in preventing transfusion-associated malaria. A combination of both methods could be considered to optimize the safety of transfusions as much as possible. Both cases presented here, illustrate this problem. Testing strategies should continue to be adapted as the number of donors coming to Germany and Europe from malaria-endemic areas is likely to increase.

We present for discussion whether antibody testing, which is falsely negative in about 40% of cases, should really be preferred to highly sensitive PCR that fails to detect small *Plasmodial* loads. Within the framework of a study, we intend to test and to compare two ELISA tests that have meanwhile been approved for donor testing with our established PCR method, as well as a new NAT assay promised by the Roche company for the end of 2023, and to decide based on these data if the implementation of two different test systems should be considered.

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Statement of Ethics

Written informed consent was obtained from participants for publication of the details of their medical case.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Carlos Jiménez Klingberg: formal analysis, writing – original draft and writing – review and editing, and coordination of testing; Lutz Pichl and Katja Konietzko: NAT testing; and Ludwig Hartmann and Bogdan Puscasu: physicians of BSD and donor care.

Data Availability Statement

All data generated or analyzed in this case report are included in this article. Further inquiries can be directed to the corresponding author.

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