Characteristics and clinical relevance of the quantitative touch-down major surface glycoprotein polymerase chain reaction in the diagnosis of Pneumocystis pneumonia

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The evaluation of quantitative polymerase chain reaction (PCR) characteristics can increase the accuracy of the laboratory diagnosis of Pneumocystis pneumonia (PCP). Between July 2008 and September 2009, 66 non-sequential prospective bronchoalveolar lavage (BAL) samples, obtained from five HIV-infected and 49 non-HIV-infected patients were investigated, using a quantitative-touch-down-PCR to determine the number of copies of major surface glycoprotein (MSG) genes of Pneumocystis jirovecii (q-TD-MSG-PCR). PCP was confirmed by microscopic observation of Pneumocystis, radio-clinical and therapeutic data in 18/54 patients. For PCP, the cut-off was 54.3 MSG copies per ml of BAL fluid. The PCR was positive in these same 18 cases and it was the only positive assay in two cases and the earliest diagnosis test in one case of PCP relapse. The likelihood positive ratio, sensitivity and specificity of the q-TD-MSG-PCR were 44, 100% and 97.7%, respectively. The Predictive Negative Value was 100% and the Predictive Positive Value of 95.5%, the intra- and inter-assay variability values were 2.7% (at more than 30 MSG copies) and 11.7% (at 10,000 MSG copies), respectively. Quantitative PCR can help diagnose PCP even in cases of low Pneumocystis load and might decrease morbidity in association with very early specific treatments.

Keywords Pneumocystis pneumonia, diagnosis, quantitative PCR, major surface glycoprotein

Introduction

Pneumocystis pneumonia (PCP) can be observed in both HIV and non-HIV patients including those with hematological malignancies, transplantation, immunosuppression, and dysimmunity and even in immunocompetent people such as severely malnourished infants [1]. The mortality rate remains high in immunocompromised patients and among HIV positive individuals in whom trimethoprim-sulfamethoxazole treatment has failed [2,3]. Generally, in patients at risk for PCP, initial diagnosis is based on non-specific clinical symptoms and typical X-ray chest images [1]. The possible co-infection of these patients with cytomegalovirus (CMV) or other microorganisms may complicate diagnosis and/or appropriate treatment [2,4]. Direct immunofluorescence assay, optical microscopy and/or cytological staining of respiratory samples are usually used to confirm PCP diagnosis due to the lack of a reliable in vitro culture system. The staining technique sensitivity (Se) varies from 60–92% for bronchoalveolar lavage (BAL) samples and from 35–78% for aspirates and induced sputum samples [5–7].
65–90% [8,9]. Therefore, it is essential to improve the Se levels of these assays for laboratory diagnosis of PCP. On the other hand, molecular biology approaches can detect microorganisms with great Se. However, some parameters of such tests require evaluation, such as the assay variability, comparative analysis of PCR results among different laboratories and the interpretation of results with low positive predictive values [10]. The requirements of further investigation to evaluate the diagnostic parameters of Polymerase Chain Reaction (PCR) techniques have also been noted elsewhere [11,12]. Several *Pneumocystis jirovecii* genes have been targeted for the PCR diagnosis of PCP [12]. Among them, the gene encoding for the major surface glycoprotein (MSG) is one of the most abundantly expressed in *Pneumocystis*, and can consequently increase the PCR diagnostic Se [13]. Moreover, PCR that targets the MSG can detect the highly variable repertoire of the MSG-gene which is composed of 50–100 different copies in each genome [14,15]. On the one hand, the most sensitive tests have been PCRs that target MSG and the large subunit mitochondrial ribosomal RNA (mtLSUrRNA) genes [12,16]. On the other hand, amplified *Pneumocystis* DNA may indicate the presence of the fungus without clinical confirmation of pneumonia. Indeed, temporary *P. jirovecii* colonization has already been reported [17]. Thus, a quantitative PCR (qPCR) has been selected, with the aim of discriminating *P. jirovecii* colonization from infection [5,18]. We have opted for a quantitative touch-down real-time PCR (q-TD-MSG-PCR) targeting the MSG of *P. jirovecii* and equipped with both a Fluorescence Resonance Energy Transfer (FRET) probe detection and an appropriate internal control [5,18–20]. The chosen method should increase the Se of PCP diagnosis with high specificity (Sp), predictive values, and low variability, which will allow physicians to monitor response to treatment.

**Material and methods**

The evaluation of the diagnostic performance of the PCR presented here is in accordance with the standards for reporting diagnostic accuracy (STARD) [21]. A summary of the q-TD-MSG-PCR standardization process is shown in Fig. 1. A total of 66 prospective non-sequential-blinded BAL fluid samples were obtained from 54 patients [29 males; 25 females; median age: 59 years; range: 8–87 years] and tested from July 2008 to September 2009 by the Laboratory of Parasitology and Mycology at the University Hospital of Grenoble, France, for detection and recognition of *P. jirovecii* (Table 1). The BAL samples were collected, stained and examined by at least two qualified microscopists, with a total 400 μl of BAL fluid analyzed by Fast Giemsa (RAL 555) and 400 μl observed after Gomori-Grocott staining on cytopsin slides.

**Quantitative touch-down major surface glycoprotein polymerase chain reaction (q-TD-MSG-PCR)**

The extraction of DNA from a pellet obtained from 2 ml of each of the BAL samples was performed with the Qiagen EZ1 DNA tissue kit and the Qiagen Bio Robot EZ1 (Qiagen SA, Courtaboeuf, France) which had an 80% efficiency. Five micro-liters of the 100 μl DNA extract solution from each sample were tested with a q-TD-MSG-PCR targeting the MSG of *P. jirovecii* and the corresponding specific primers (1 μM each). Probes (0.2 μM each), 5 mM MgCl₂, and 1 U of uracil-DNA glycosylase were used to obtain a final volume of 20 μl per capillary tube [5,19]. Each assay was repeated twice. The mix LightCycler (LC) Fast Start DNA hybridization probes (Roche, Meylan, France) was used with the LightCycler 2.0 (Roche, Meylan, France). Specific primers (0.5 μM each) and probes (0.2 μM each) that targeted mouse galactose-1-phosphate-uridyl transferase were employed as internal controls [5]. The q-TD-MSG-PCR was carried out with FRET hybridization probes with fluorescence readings at 640 nm for the *Pneumocystis* probe and 705 nm for the internal controls. The quantification began after the touch-down step. The standard curve was obtained with plasmids containing 10⁴, 10³, 10² and 10¹ MSG copies per capillary. The results were expressed as the means of both measures in the number of MSG copies/ml of BAL fluid. To standardize the PCR for PCP diagnosis, the intra- and inter-assay variability values were assessed. The dynamic range of q-TD-MSG-PCR was evaluated between 10¹ and 10⁸ MSG copies of *P. jirovecii* per capillary. *P. jirovecii* variants were evaluated by their respective melting peak analyses.

**Determination of the cut-offs**

For the characterization of the diagnostic variables of the PCR, the cut-off for PCP was determined by both the analysis of the ROC curve and the best addition of Yule’s Q Coefficient and Youden’s index.

**Diagnostic criteria of Pneumocystis pneumonia**

Clinically suspected PCP patients. PCP has a broad spectrum of clinical and paraclinical signs [1]. It was suspected when the patient under antibiotic signs had symptoms of acute respiratory illness with one or more of the following features; fever, dry cough, diffuse interstitial infiltrates (chest radiography), and ground glass attenuation (tomodensitometry).

Confirmed PCP patients. The gold standard used in this study was both the clinical suspicion of PCP associated with the presence of *P. jirovecii* detected by direct microscopic analysis of Giemsa and/or Gomori-Grocott stained samples.
and/or if microscopic examination was negative, a favorable outcome under specific treatment for PCP.

**Prophylaxis and treatment**

Both the prophylaxis and curative therapy used were in accordance with the usual guidelines for PCP treatment, e.g., (a) trimethoprim-sulfamethoxazole or pentamidine was prescribed for prophylactic purpose and as curative treatment; (b) atovaquone was given as curative treatment in case adverse effects caused by trimethoprim-sulfamethoxazole, and (c) oral or intravenous therapy was given to patients with either mild symptoms or significant hypoxemia. It should be noted that the patients did not systematically receive prophylactic therapy.

**Statistical analyses**

The Mann-Whitney U test was performed with the different variables to obtain a significant or insignificant difference between the suspected and proven PCP groups. For analysis, hematological malignancy and corticoid-treated solid tumor pathology factors were grouped. \( P \) value < 0.05 was considered significant. The Confidence Interval (95% CI) was evaluated for per cent of data. The diagnostic accuracy (ACC) and precision (PRE) were defined as follows:

\[
ACC = \frac{(TP + TN)}{(P + N)};
\]

\[
PRE = \frac{TP}{(TP + FP)};
\]

in which TP = true positive; TN = true negative; P = number of positive and N = number of negative.
Table 1  Characteristics and risk factors in suspected and confirmed PCP patients.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Suspected</th>
<th>Confirmed</th>
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<tbody>
<tr>
<td>Patients</td>
<td>54</td>
<td>36</td>
<td>18</td>
</tr>
<tr>
<td>Analyzed bronchoalveolar lavage</td>
<td>66</td>
<td>44</td>
<td>22</td>
</tr>
<tr>
<td>Male/female</td>
<td>29/25</td>
<td>21/15</td>
<td>28/10</td>
</tr>
<tr>
<td>Haematological malignancies and corticoid treated-solid tumor pathology ($P = 0.0114$)</td>
<td>37/54</td>
<td>24/36</td>
<td>13/18</td>
</tr>
<tr>
<td>Solid transplantation</td>
<td>13/54</td>
<td>12/36</td>
<td>1/18</td>
</tr>
<tr>
<td>HIV+</td>
<td>5/54</td>
<td>2/36</td>
<td>3/18</td>
</tr>
<tr>
<td>Neutrophils G/l, median [range] ($P = 0.0465$)</td>
<td>3.3 [0–30.7]</td>
<td>4.9 [0–30.7]</td>
<td>1.9 [0–15.1]</td>
</tr>
<tr>
<td>Lymphocytes G/l, median [range] ($P = 0.0416$)</td>
<td>0.95 [0–9.5]</td>
<td>1.1 [0–3.5]</td>
<td>0.6 [0.1–9.5]</td>
</tr>
</tbody>
</table>

PCP, Pneumocystis pneumonia; SD, standard deviation; $P$ values obtained by Mann-Whitney U test between suspected and confirmed PCP patients.

Results

Characteristics in suspected and confirmed Pneumocystis pneumonia patients

Table 1 provides the characteristics of suspected and confirmed PCP patients. Haematological malignancy, corticoid treated-solid tumor pathology ($P = 0.0114$) together with neutrophil ($P = 0.0465$) and lymphocyte counts ($P = 0.0416$) varied significantly between the suspected and confirmed PCP patients (Mann-Whitney U test). The other factors, such as age and sex, did not significantly differ from one group to another (Table 1). Among the confirmed PCP cases, 3/18 patients (16.7%) were HIV positive, 95% CI [6–40%].

General data from enrolled patients

Table 2 presents the clinical and paraclinical data from the suspected (and unconfirmed) and confirmed PCP groups. Among the clinically suspected cases, the study revealed: (a) Seventeen patients with infectious (bacterial, viral and fungal) pneumonia other than PCP, (b) three patients with interstitial pneumonia associated with autoimmune disorders, (c) three patients with idiopathic pulmonary fibrosis, (d) two patients with pulmonary arterial hypertension, and (e) 11 patients with other forms of pneumopathy (pulmonary emphysema, bronchopneumonia, bronchorrhea, pneumothorax, progression of broncho-alveolar carcinoma, etc.). Persistent fever and hypoxemia were significantly different from one group to another, as indicated by the Mann-Whitney U test (Table 2). The other clinical or paraclinical records did not demonstrate significant differences in the two groups.

Characteristics of the laboratory tests used for PCP diagnosis

Table 3 partly summarizes the characteristics of the three laboratory tests used for PCP diagnosis. In this study, the chosen cut-off for *P. jirovecii* infection optimized the best Se with an optimal Sp (Fig. 2D). After determination of this cut-off by the ROC curve (54.3 MSG copies per ml of BAL fluid), the obtained diagnostic Se, Sp, Predictive Positive and Predictive Negative values are shown in Table 3. The q-TD-MSG-PCR was the most sensitive, discriminative and accurate assay in the diagnosis of PCP, while the staining techniques were the most specific. The likelihood positive ratio of the selected cut-off was 44 for the q-TD-MSG-PCR-based diagnosis whereas the likelihood negative ratio was zero. Table 3 also highlights both the characteristics of the Gomori-Grocott and Fast Giemsa diagnosis methods used in our study, and the area under the ROC curve obtained for each assay.

The results of these laboratory assays are linked to PCP, as reflected by the Spearman’s rank correlation coefficient (data not shown), the Yule’s Q coefficient (Table 3) and the significant differences between the confirmed and suspected PCP groups (Mann-Whitney U Test; $P = 0.0001$).

The characterization of the q-TD-MSG-PCR was continued by: (a) The determination of the intra- and inter-assay variability values, as shown in Fig. 2A and 2B; (b) the determination of the dynamic range of the q-TD-MSG-PCR that covered 7 log units, as presented in Fig. 2C;
The analysis of the variation, Se and 1-Sp by the ROC curve (area under the curve = 0.986; [95% CI, from 0.919–0.996]), as shown in Fig. 2D; (d) the diagnostic accuracy as shown in Table 3; and (e) the detection of MSG gene variants thanks to analysis of the melting temperature (Tm). One example (Fig. 2E) shows the melting temperature accuracy as shown in Table 3; and (e) the detection of MSG gene variants thanks to analysis of the melting temperature (Tm). One example (Fig. 2E) shows the melting temperatures obtained from three different isolates of *P. jirovecii*. A given Tm was arbitrarily considered dissimilar if the difference was > 0.5°C.

**Clinical characteristics of PCP patients with regards to laboratory results**

Table 4 illustrates clinical, laboratory and radiological data from 18 patients with PCP and one with CMV infection. Among the 18 PCP patients, 11 had hematological malignancies (61%, 95% CI [38–80%]). As to confirmed PCP cases that fulfilled the diagnostic criteria, 3/18 patients (16.7%) were HIV positive, 95% CI [6–40%]. The overall mortality rate among PCP patients was 6/18 (33%, 95% CI [16–57%]) versus 4/11 (36%, 95% CI [15–65%]) among those with only hematological malignancies. Some of the patients were subjects of surveillance studies from whom two to four BAL samples were collected. In patient 4, who had an intolerance to trimethoprim-sulfamethoxazole, PCR was the earliest assay (Day 0) performed in relation with a PCP relapse. Under treatment pressure, *P. jirovecii* was not microscopically observed with Giemsa and Gomori-Grocott staining performed at Day 27, although numerous MSG copies were measured by PCR. In patients 17 and 18, the staining methods were negative for *P. jirovecii* trophozoite and cyst detection in BAL fluids. For this individual, the PCR results were positive despite the pentamidine prophylaxis and the trimethoprim-sulfamethoxazole treatment that had been initiated a week before the BAL fluid examination. After the episode of clinical severe pneumocystosis, patient 17 had a favorable outcome but thereafter developed multifactor pulmonary fibrosis and died of terminal respiratory failure two weeks later as a result of immunodeficiency caused by treatment of a thoracic neuroblastoma.

For patient 18, during an episode of mild pneumocystosis, PCR results were positive and after a week of curative trimethoprim-sulfamethoxazole treatment, a marked decrease in the number of the pulmonary interstitial infiltrates was noted in a context of immunodeficiency due to the treatment of acute myeloid leukemia. Patient 19 suffered from CMV pneumonia, although the clinical evolution was not favorable under CMV-specific treatment. For this patient with possible PCP, PCR was positive but the pneumocystosis was not proven due to the lack of a specific treatment (see diagnostic criteria). Thus, the data was considered to be indicative of a false positive for the determination of the diagnostic characteristics of the technique under study.

**Discussion**

In the laboratory diagnosis of PCP, a few technical and biological factors can explain the robustness and reliability of the q-TD-MSG-PCR. Since the same DNA-extraction method was used for each BAL sample, the possible PCR inhibition may be uniform in all the samples since the crossing points of the internal controls had been stable throughout the study. Moreover, PCR inhibition due to the DNA extraction method can be very low, as no false negative results were observed. Thus, an absolute quantification of MSG gene copies may be done. The chosen touch-down phase may increase the analytical Se and Sp of the assay [19,22]. Then, the FRET probes utilized may increase the Sp of PCR diagnosis with no loss of analytical Se. Finally, the q-TD-MSG-PCR can detect the highly variable repertoire of the MSG-gene [14,15]. This variability was overcome due to a base degenerated-MSG primer and probe. This phenomenon is reflected in the melting peak analysis that allowed us to appreciate the genetic variability of *P. jirovecii* isolates.
Fig. 2  Standardization of the PCR (q-TD-MSG-PCR). (A) Intra-assay variation coefficients in q-TD-MSG-PCR experiments. The intra-assay variability (Y-axis) is represented with empty circles. It was calculated from the Logarithm of the MSG copies per capillary plus 1, from patient samples with positive duplicate data (X-axis) in the range of MSG copies/per capillary ≥ 30 (n = 14; mean: 2.7%; standard deviation: 2.9%). The solid triangles and the vertical bars are read in the X-axis and represent the measure plus one SD, respectively. (B) Inter-assay variability in q-TD-MSG-PCR. Left side of the Y-axis: The Figure shows by the means of empty rectangles the inter-assay variability from 20 independent experiments performed with the standard curves. Right side of the Y-axis: the solid rhombuses and the horizontal lines represent the logarithm of the MSG copies and ± one SD. The X-axis represents the number of MSG copies tested per capillary. The q-TD-MSG-PCR inter-assay variability increased in inversely proportional way compared to the MSG-copy load in the range of 100–10,000 copies per capillary, and remained acceptable as a laboratory test for the diagnosis of *Pneumocystis* pneumonia in clinical suspected patients. The 10-copy-per-capillary load was detected in 14/20 experiments (70%; 95% CI, from 47.8–85.4%). The limit of detection (LOD) was then 32.7-copy-per-capillary as calculated from probit values. (C) Dynamic range of q-TD-MSG-PCR. The Y-axis indicates the crossing point and the X-axis the concentration expressed by the logarithm of the number of MSG copies tested. Dynamic range of quantification was from 10–10^7^ copies per capillary: that is 7-log units. This is indicated by the line equation in this interval with a significant value of R^2^ (goodness-of-fit of linear regression). (D) Receiving Operating Characteristic (ROC) curve of q-TD-MSG-PCR. All the data from the 66 BAL fluids is necessary for the elaboration of the ROC curve. An arrow indicates the best cut-off (54.3 MSG copies/ml of BAL fluid) that was also obtained also by the best addition of Youden’s index and Yule’s Q Coefficient (see Table 3). The cut-off with 100% Sp was 66.800 MSG copies/ml of BAL fluid. The discriminative power of the test was very good since the area under the ROC curve was 0.986. (E) Detection of MSG-isolate variants of *Pneumocystis jirovecii*. Y-axis: negative derivative of the fluorescence with respect to temperature. X-axis: temperature (°C). In the follow-up of three immunocompromised patients with *Pneumocystis* pneumonia, q-TD-MSG-PCR detected 3 MSG variants with different melting peaks Mp1, Mp2 and Mp3 indicated by the arrows: the corresponding melting temperatures Tm1, Tm2 and Tm3 are read in the X-axis.
For a fine analysis of this variability, MSG-gene sequencing of the isolates may be necessary [14]. The approximate genetic variability may account for the q-TD-MSG-PCR high Se and reflects the nature of the \textit{P. jirovecii} variants in human samples. The q-PCR can evaluate the MSG copies of \textit{P. jirovecii}, in contrast with the staining techniques that detect the fungus. Indeed, in one BAL sample from patient 4, who was under trimethoprim-sulfamethoxazole treatment, numerous MSG copies were quantified although the fungus was not identified on different slides.

Although a binary-PCR result may be useful for medical screening, q-PCR is required for laboratory diagnosis, as well as for the determination of an optimal cut-off value for PCP, as a result of the fact that a PCR-based diagnosis can have low positive predictive value [5,10,16,20]. In semi or quantitative PCR, low-predictive values have been obtained when the gold standard is a laboratory assay [20,23]. From the practical point of view, an infection cut-off discriminates between suspected and confirmed PCP patients. This has also been found between the patients with low and high probability of PCP by another quantitative PCR that targets the mtLSUrRNA gene of \textit{P. jirovecii} [16]. Then, the rationale for laboratory diagnosis may be the determination of the cut-off value for PCP, which has to take into account the patient’s clinical, immunological and radiological data. A colonized immunocompetent person or a hospitalized-HIV-infected patient may not present clinical manifestation of PCP [24]. The infection cut-off can discriminate between \textit{Pneumocystis} colonization and infection in an appropriate clinical context. The high value of the area under the ROC curve supports this observation.

In our study, the analysis of the ROC curve suggests a close relationship among the diagnostic Se, Sp and cut-off value of a test. Such relationship has also been reported by Larsen et al. [18] who employed the same type of PCR, except that DNA was extracted from oral washes of HIV-infected PCP patients and from BAL samples. In the study of Alanio et al. [16] with another quantitative PCR system, this kind of relationship was also found with the lower and the upper cut-off values. Therefore, a combination of a
quantification procedure and an optimal cut-off may lead to a 100% diagnostic Se with a good Sp as seen in the present study.

*P. jirovecii* can transiently colonize healthy people since this microorganism is usually complete cleared [17]. In immunocompromised, as well as in healthy people, the colonization by *Pneumocystis* may act as a reservoir for infection and exacerbate other pulmonary diseases [13,25,26]. Immunocompromised patients, with no clinical signs of pneumonia and a positive q-TD-MSG-PCR test, can be assumed to be colonized by *P. jirovecii*, and may be monitored by the serum (1→3)-beta-D-glucan content for an eventual PCP [8,9]. Immunocompromised patients, with both clinical signs of pneumonia and a positive q-TD-MSG-PCR test, can be assumed to be infected by *P. jirovecii*. Our results suggest that the notions of infection and colonization by *P. jirovecii* are relative and may depend on the PCP clinical manifestation, the patient’s immune status, associated co-infections, the level of intolerance to treatment and the extent of vital risk. This was observed in patient 17, affected by a neuroblastoma associated with treated PCP before the PCR test, and in the studies by Larsen et al. [18,19]. Such high diagnostic sensitivity with a good Predictive Positive Value may also be useful in patients with hematological malignancies and possible low fungus load associated with PCP, as observed in patient 18, who had acute myeloid leukemia. In some cases, the improvement of clinical and paraclinical signs of pneumonia under specific therapy can provide the answer in association with positive q-TD-MSG-PCR data. The q-TD-MSG-PCR method may have one of the highest Se of all laboratory assays used to diagnose PCP (see Table 5) [5,12,19].

The high negative predictive value of q-TD-MSG-PCR suggests that a negative result from BAL samples rules out the possibility of *Pneumocystis* pneumonia, as observed in our study and others [20]. Comparable diagnostic Se and Sp for PCP have been observed with this test in various laboratories and may represent a path towards the standardization and optimizations of PCR assays [5,16,19,20]. The inter-laboratory results with PCR are comparable, in spite of the use of different internal controls and software [5,19]. This path towards standardization has been reached with one PCR technique, i.e., q-TD-MSG-PCR. However, the recent paper by White et al. [27] suggests that this can be achieved with several types of PCR. This step should also be crossed with multiple quantitative *Pneumocystis* PCR [16,23,28–30]. In the present study, we briefly compared the quantitative PCR with the crossing point data from a commercially-available kit that targets mtLSU rRNA gene of *P. jirovecii* (FXGMT: RESP (Asp+): Myconostica Ltd (Manchester, UK)) with a good correlation ($r = 0.959; P = 0.0006$, data not shown). Only one major difference was observed since FXGMT-*Pneumocystis* PCR result was negative in the case of a PCP relapse in patient 4 (data not shown). To our knowledge, the consensus definitions for the biological diagnosis of PCP do not exist yet. Quantitative PCR might be admitted in this future consensus as a diagnostic tool, together with other direct or indirect microscopic observations. When an immunocompromised patient is followed up because of a high risk for PCP, an optimally standardized q-PCR with high Se is useful to quantify DNA from *P. jirovecii*. In confirmed PCP patients, the morbidity may be decreased thanks to q-PCR and early specific treatment. Moreover, the high negative predictive value of q-PCR may avoid useless treatment in suspected patients.

Although it is well known that the clinical signs are not specific to PCP, we noted that both persistent fever and hypoxemia may have significantly different values between suspected- and confirmed-PCP groups of patients. In our study, the main risk factor for *Pneumocystis* pneumonia was hematological malignancies and corticoid treated-solid tumor as has been reported elsewhere [31]. This may be due to the absence of primary prophylaxis and intensive therapy against hematological forms of cancer. Likewise, our lymphocyte count data support the finding that lymphopenia is a risk factor for PCP [32]. The course of PCP and the mortality rate were not favorable in HIV-negative patients, as has been reported in other studies [33]. However, Fisk et al. [3] noted that this might be true for HIV-infected patients in whom trimethoprim-sulphamethoxazole treatment has failed. We did

### Table 5 Specific diagnosis of PCP by quantitative PCR: comparative performances.

<table>
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<tr>
<th>Study</th>
<th>Target gene</th>
<th>Se %</th>
<th>Sp %</th>
<th>PPV %</th>
<th>NPV %</th>
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<tr>
<td>Rohner [23]</td>
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<td>94.3</td>
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</tbody>
</table>

* These values were calculated from data presented in the respective publication. Beta-tubulin gene; Se, Sensitivity; Sp, Specificity; PPV, Predictive positive value; NPV, predictive negative value; DHPS, Dihydropteroate synthetase gene; DHFR2, Dihydrofolate reductase gene; HSP 70, heat shock protein 70 gene; ITS-2, Internal transcribed spacer region; Kex-1, serine endoprotease gene; 5.8 S rRNA, 5.8 S ribosomal gene; mtLSU rRNA, mitochondrial large subunit of ribosomal RNA; MSG, Major Surface glycoprotein gene; (T), using TAMRA as reporter dye.

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not observe such results since our study included only three HIV-positive patients with PCP. Under highly active antiretroviral therapy, the overall mortality rate among those patients was 11.3% but this rate varied in terms of the disease severity, ranging from 3.7–49.1% [31]. The high-mortality rate and/or intolerance to PCP treatment can justify the use of a q-PCR with high Se and high PPV.

Conclusion

The q-TD-MSG-PCR has a good diagnostic value independent of the disease prevalence and is rather fast. Quantitative PCR targeting MSG gene of \( \text{P. jirovecii} \) could be used as an early, discriminating, and accurate tool to diagnose PCP even in cases of low \( \text{Pneumocystis} \) load. Used in association with a very early specific treatment, it may help decrease morbidity.

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