Clinical significance of quantifying *Pneumocystis jirovecii* DNA using real-time PCR in bronchoalveolar lavage fluid from immunocompromised patients

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Running title: Quantifying *Pneumocystis jirovecii* DNA

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Abstract

Quantitative PCR (qPCR) is more sensitive than microscopy for detecting *Pneumocystis jirovecii* in bronchoalveolar lavage (BAL) fluid. We therefore developed a qPCR assay and compared the results with routine immunofluorescence assay (IFA) and clinical data. The assay included automated DNA extraction, amplification of the mtLSU gene and internal control, and quantification of copy numbers with the help of a plasmid clone. We studied 353 consecutive BAL fluids obtained for investigation of unexplained fever and/or pneumonia in 287 immunocompromised patients. No qPCR inhibition was observed. Seventeen (5%) samples were both IFA and qPCR positive, 63 (18%) were IFA-negative and qPCR-positive, and 273 (77%) were both IFA and qPCR negative. The copy number was significantly higher for IFA-positive/qPCR-positive than for IFA-negative/qPCR-positive samples (4.2±1.2 vs. 1.1±1.1 log10 copies/µl, p<10^{-4}). With IFA as the standard, the qPCR assay sensitivity was 100% for ≥2.6 log10 copies/µl and the specificity was 100% for ≥ 4 log10 copies/µl. Since qPCR results were not available at the time of decision making, these findings did not trigger co-trimoxazole therapy. Patients with systemic inflammatory diseases and IFA-negative/qPCR-positive BAL had a worse one-year survival than those with IFA-negative/qPCR-negative results (p<10^{-3}), in contrast with solid organ transplant recipients (p=0.88) and patients with hematological malignancy (p=0.26). Quantifying *P. jirovecii* DNA in BAL fluids independently of IFA positivity should be integrated in the investigation of pneumonia in immunocompromised patients. The relevant threshold remains to be determined and may vary according to the underlying disease.

**Keywords:** *Pneumocystis jirovecii*, real-time quantitative PCR, bronchoalveolar lavage.
The laboratory diagnosis of *Pneumocystis* pneumonia, referred to as PcP, and caused by the opportunistic fungal pathogen *Pneumocystis jirovecii*, still relies on tinctorial and/or immunofluorescent staining of bronchoalveolar lavage (BAL) fluid samples (27). In contrast to microscopy, nucleic acid amplification tests can overcome the difficulties of microscopic examination (27). Several previously reported PCR studies have used nested PCR with a final endpoint reading (16, 20). This format is not intended to give quantitative results and is prone to contamination with previously amplified products leading to false positive results. In contrast, real time quantitative PCR (qPCR) dramatically reduces the risk of false positive because of the close-tube nature of the amplification process (6) and the resulting data are quantitative if guidelines for interpretation qPCR results are followed (7).

Several qPCR assays have already been published and have shown that qPCR is more sensitive than microscopy (1-3, 5, 10-12, 14, 15, 17, 18, 22, 26). This raises the issue of the clinical significance of *P. jirovecii* DNA detection for explaining the symptoms observed (24, 25). Quantitative PCR data can also address the issue of the correlation between tissue burden and outcome (16). In particular, we questioned the use of microscopically detectable *P. jirovecii* cysts as a threshold for therapeutic decision making, especially if extended to all patients whatever their risk factors for developing PcP. Therefore, we developed a qPCR assay and tested all the bronchoalveolar lavage (BAL) fluids received by our laboratory without selection of the patients from whom samples were obtained. The qPCR results were compared with immunofluorescence assay (IFA) results, in light of medical information including underlying diseases and outcome.

**MATERIALS AND METHODS**

**BAL fluid sampling and conventional diagnosis of PcP**
This is a retrospective single-center, hospital-based cohort study on all patients who underwent BAL to investigate the cause of pneumonia and/or unexplained fever from January 1st, 2006 to December 31st, 2007. The study was approved by the “Comité de Protection des Personnes, Ile de France”. Since the qPCR assay was performed blind on stored samples, the results did not alter therapeutic management of the patients.

BAL was performed with three washes (Wash 1 to 3) of 50 ml sterile saline solution. Aliquots of the washes were dispatched to the microbiology and pathology laboratories. Upon arrival in the laboratory, at least 10 slides were prepared each using 200 µl of Wash 2. The routine procedure included May-Grünwald Giemsa (MGG) staining (RAL-555, Martillac, France), methenamine silver staining (MSS), and an indirect immunofluorescence assay (IFA) (Monofluo kit P. jirovecii, Biorad, Marnes la Coquette, France). Information recorded included the total volume of BAL fluid recovered, lung X-ray or CT-scan conclusion, demographics, underlying conditions at the time of the BAL procedure, outcome at one year as well as anti- \textit{Pneumocystis} prophylaxis at the time of BAL procedure and anti- \textit{Pneumocystis} therapy prescribed after results of the BAL were known.

\textbf{DNA extraction from BAL fluids.}

From the BAL fluid Wash 2 remaining after microscopy and microbiological cultures, 1.5 mL of BAL fluid was centrifuged at 10,000 g for 10 minutes. Supernatant was aspirated and the cell pellet was stored in 200 µl of the washing solution at -40°C until further processing. After thawing, DNA was extracted using the QIAmp DNA mini kit (Qiagen, Courtaboeuf, France) according to the manufacturer's instructions, except that total DNA was eluted from the spin columns with 50 µl of elution buffer in order to increase the DNA concentration. Five µl of this DNA extract were used per qPCR reaction.

\textbf{Pneumocystis jirovecii qPCR assay.}
Two primers (forward: PNC-LSU3: 5’-TGGTAAGTAGGAAATACAAATCGG-3’; reverse: PNC-LSU4: 5’-ACTCCCTCGAGATATTCAGTGC-3’) and a pair of hybridization probes (PNC-LSU5 5’LCRed640 3’Ph labeled: 5’-TTCGCAGAAAACCAGCTATATCCTAGT-3’; PNC-LSU6 3’FITC labeled: 5’-AGAGGAATACAAATTGCCCCAAAACAA-3’) were selected to amplify and to detect a conserved 152-bp region of the mitochondrial large sub-unit rRNA gene (LSU) of *P. jirovecii* using real-time PCR (Genbank accession number: AJ608260). The amplification was carried out in a LightCycler 1.5 instrument (Roche Diagnostics, Meylan, France). PCR was set up in a final volume of 20 µl with the Faststart DNA Master Hybridization Probes Kit (Roche Diagnostics, Meylan, France), 4 mM MgCl₂, each primer and probe (Sigma, Paris, France) at a concentration of 0.5 µM and 0.25 µM respectively, and 0.25 µl of uracil-DNA-glycosylase (UDG) (Biolabs, Courtaboeuf, France). The reaction mixture was initially incubated for 1 min at 50°C followed by a 8-min step at 95°C. Amplification was performed for 50 cycles of denaturation (95°C for 10 s; ramp rate, 20°C/s), annealing (60°C for 10 s; ramp rate, 20°C/s), and extension (72°C for 15 s; ramp rate, 20°C/s). Results were considered as positive when a significant fluorescent signal above the base-line was detected as determined by the second-derivative algorithm method and were expressed as quantification cycle (Cq) values. During each run, a *P. jirovecii* DNA positive control and the elution buffer for DNA extraction as a negative control were used. Residual amplification inhibitory effect in the DNA extract was tested by using a universal internal standard as previously described (8) and as adapted for the LightCycler 1.5 instrument.

A 299 bp fragment of the *LSU* gene including the targeted PCR fragment was cloned into a pUC57 plasmid (Ecole de Biologie Industrielle, Cergy-Pontoise, France) and its concentration was measured using a fluorometer and the corresponding copy number was calculated. A ten-fold serial dilution series of this plasmid clone ranging from 1 to 10¹¹ copies/ml was used to construct the standard curve. Cq values in each dilution were measured.
in duplicate in three independent runs and were plotted against the logarithm of their initial template copy numbers. Results were expressed as copy/µl for each DNA extract of positive sample determined against the standard curve.

Statistical methods

Means and standard deviations (SD) are shown when distributions were confirmed as normal or after log10 transformation when needed. The comparisons used Fisher's exact test for categorical variables, and the t-test or one-way ANOVA test for continuous variables using Prism 4.0 (GraphPAD Software). Overall survival at 12 months was calculated using Kaplan Meier analysis from the date of the first BAL to the one-year follow-up or death from any cause. When multiple BALs with different qPCR results were available for one patient, the first qPCR positive BAL was used for the analysis.

RESULTS

Validation of the qPCR assay

Our qPCR assay could systematically detect the dilution containing 1 copy of plasmid per µl leading to a detection sensitivity of at least 5 copies per PCR reaction. Quantification was linear over a ten-magnitude order and the standard curve was generated with a high coefficient of determination \( (R^2=0.999) \). The mean overall coefficient of variation (CV) of the \( \text{Cq} \) values was 1.5% (range: 0.5-3.5) (Table 1). For the \( P. \ jirovecii \) DNA positive control used, the mean \( \text{Cq} \) on 20 runs was 32.41+/−0.20 (CV= 0.62%).

Analysis of BAL specimens

A total of 378 consecutive BAL specimens were collected in our laboratory during the study period, of which 353 had sufficient volume left for qPCR analysis after routine testing.
No PCR inhibition was detected and a correct amplification of the internal control was observed in each sample (mean Cq of the internal control = 37.1 ± 1.1), allowing analysis of the qPCR results. Of these 353 samples, 10 (2.8%), 16 (4.5%), and 17 (4.8%) were microscopically positive for *P. jirovecii* using MSS, MGG, and IFA, respectively. All the MSS positive samples were MGG positive, and all the MGG positive were IFA positive. The 17 IFA-positive samples were all qPCR-positive.

Overall, three groups of samples were thus available for analysis: IFA-positive/qPCR-positive samples (n=17, 4.8%), IFA-negative/qPCR-positive samples (n=63, 17.8%), and IFA-negative/qPCR-negative samples (n=273, 77.3%). Since the recovered BAL volume could interfere with qPCR quantification, we checked that it did not significantly differ between the three groups (Table 2, p>0.05). The copy number was markedly (more than three logs in magnitude) higher for IFA-positive/qPCR-positive samples than for IFA-negative/qPCR-positive samples (4.2±1.2 vs. 1.1±1.1 log10 copies/µl, p<10^{-4}) (Table 2). With IFA as the standard, the qPCR assay sensitivity was found to be 100% for samples containing ≥2.6 log10 copies/µl and the specificity was 100% for those with ≥4.0 log10 copies/µl. Among the 13 samples containing ≥2.6 log10 and <4.0 log10 copies/µl, 8 were IFA-negative/qPCR-positive. These 8 samples were from 8 patients who were not given cotrimoxazole and 5 of them died at day 6 (solid cancer), day 120 (HIV-positive patient), day 197 (acute myeloid leukemia), day 289 (acute lymphoid leukemia) and day 353 (HIV-positive patient) from the day of the first BAL.

The presence of diffuse pulmonary lesions with no localized/focal lesions on chest X-ray or CT-scan imaging was unequally distributed among the three groups (Table 2). However, although there were more diffuse lesions in the IFA-negative/qPCR-positive group than in the IFA-negative/qPCR-negative one, the difference was not statistically significant (p=0.24). In contrast, the difference in anti-PcP prophylaxis at the time of the BAL was highly significant with almost no prophylaxis in patients with qPCR-positive samples (Table 2).
Analysis according to underlying diseases

The main underlying risk factors/diseases were divided into 6 groups. When several risk factors were present, the samples were classified in the highest risk factor category in the following decreasing order: HIV positivity; acute leukemia; chronic lymphoproliferative disorder; solid organ transplantation; systemic inflammatory diseases; solid tumor; and other underlying disease.

For the 46 samples from HIV-positive patients, both the CD4+ T cell count and anti-PcP prophylaxis predicted most of the results. Among the 16 samples from HIV positive patients with a CD4+ T cell count ≥ 200 cells/µl, only one (6.2%) was qPCR-positive (Table 3). The 19 IFA-negative/qPCR-negative samples recovered from patients with CD4+ T cell count < 200 cells/µl, 15 (78.9%) corresponded to patients under anti-PcP prophylaxis.

Among the 147 samples from the hematological population, only 6 (4.1%) were IFA-positive/qPCR-positive whereas 24 (16.3%) were IFA-negative/qPCR-positive (Table 3). Similarly, only 4% (2/57) and 2% (1/53) of samples were IFA-positive/qPCR-positive whereas 18% (10/57) and 30% (16/53) were IFA-positive/qPCR-positive samples for solid organ transplant (SOT) recipients and patients with systemic inflammatory diseases (7 vasculitis, 14 inflammatory rheumatisms, 6 glomerulonephritis, and 20 other diseases), respectively (Table 3). A qPCR-positive result was also frequent in samples from solid cancer patients (7/18, 39%). The two IFA-negative/qPCR-positive samples from the group with none of the above risk factors were from a patient with influenza virus infection and type 2 diabetes, and from a patient with chronic obstructive pulmonary disease.

Outcome analysis

Co-trimoxazole at curative dose was given only to the patients with IFA-positive
results and to 6 other patients based on clinical suspicion [3/63 (5%) in the IFA-negative/qPCR-positive group and 3/273 (1%) in the IFA-negative/qPCR-negative group].

There was no significant difference in survival at one year between IFA-negative/qPCR-positive (n=22) and IFA-negative/qPCR-negative (n=74) patients with hematological diseases (p=0.26; Fig. 1 A). Similar results were observed for the SOT recipients with no significant difference (p=0.88) between IFA-negative/qPCR-positive (n=8) and IFA-negative/qPCR-negative (n=35) patients (data not shown). In contrast, one-year survival was significantly worse (p<10^{-3}; Fig. 1 B) for IFA-negative/qPCR-positive (n=14) than for IFA-negative/qPCR-negative patients (n=25) with systemic inflammatory diseases. Of note, the mean age (in years ±SD) was not statistically different between the IFA-negative/qPCR-positive and the IFA-negative/qPCR-negative patients (63±10 vs. 58±13, p=0.22).

Additional BAL samples (n=66) were obtained from 52 patients (mean [range] =2 [2-6] samples/patient) with a mean interval of 43.5 days (range: 1-652 days). Thirty-six IFA-negative/qPCR-negative patients remained IFA-negative/qPCR-negative. Five qPCR-positive patients remained qPCR-positive in an interval <1 month, with a decrease of the fungal load when given co-trimoxazole (3 patients). Three patients previously IFA-positive/qPCR-positive became IFA-negative/qPCR-negative >2 months after co-trimoxazole therapy. Six patients who were IFA-negative/qPCR-positive became IFA-negative/qPCR-negative >2 months without any known specific therapy. Two IFA-negative/qPCR-negative patients became IFA-positive/qPCR-positive within 2 and 12 months, respectively.

DISCUSSION

The present qPCR assay proved to be a reliable tool for quantifying *P. jirovecii* DNA in BAL fluid samples. We paid attention to avoid false positive samples and unreliable
quantification by checking the efficiency of the reaction using an internal control. Under these conditions, all immunofluorescence positive BAL fluid samples (17/353; 4.8%) were found to be qPCR positive and 17.8% (63/353) were qPCR positive only.

Our results confirm the higher sensitivity of qPCR over microscopy as already reported by many authors using qPCR assays. However, there are marked differences among studies, which prevents direct comparison. For instance, the target chosen for amplification was either a multicopy gene [major surface glycoprotein MSG (10, 11, 17, 18), ribosomal DNA (1, 3, 12, 14, 22)], or a single copy gene (3, 5, 15, 18, 26). The use of UNG for preventing cross-contamination has only been reported in the present study. An internal control has been effective (1, 5, 11, 14, 17), omitted (2, 3, 12, 15, 18, 22, 26), or inadequately chosen (10). Results have been expressed in copy number using a plasmid clone (2, 3, 5, 12, 15, 17, 22) or as Cq values (1, 10, 11, 14, 18, 26). Additionally, cross-study comparison is difficult when the type of clinical specimen sampled is different. Most publications have used BAL fluids only (2, 3, 5, 10, 11, 15, 18, 22, 26), but others have included mixed sputa and BAL (1, 14) or sputa and/or oral washes (12, 17). Even though oral washes or sputum samples can be used for \textit{P. jirovecii} detection, we used the BAL fluids only to keep the quantification consistent.

Most authors rely on immunofluorescence assay and lung imaging to evaluate the performance of their PCR assays (4, 20). Thus, PCR positive/immunofluorescence negative samples that are seen in cases devoid of suggestive lung lesions are considered false positive results, and therefore decrease the overall positive predictive value of PCR assays. In contrast, the negative predictive value is high since samples with positive immunofluorescence and negative PCR results are extremely rare, even absent as in the present study. If IFA positivity is the criterion to define PcP, the sensitivity of our qPCR assay was 100% for samples with \( \geq 2.6 \, \text{log}_{10} \, \text{copies/µl} \) and the specificity was 100% for samples with \( \geq 4 \, \text{log}_{10} \, \text{copies/µl} \). Thus, for eight IFA-negative patients, a specific therapy was not started although they had
positive qPCR results between our thresholds of sensitivity and specificity. This suggests that IFA positivity cannot be the sole criterion to start specific therapy. Imaging cannot help in the IFA-negative/qPCR-positive cases since the lung lesions observed were not different from those seen in the IFA-negative/qPCR-negative group. Also, a strategy based mainly on clinical and radiological signs does not take into account the fungal load which is yet an important issue in other infectious diseases. In hematology for instance, the treatment of CMV disease is started above a consensual threshold (19), and for infection with *Toxoplasma gondii*, the recommendation is to start therapy as soon as a blood PCR result is positive (21).

Therapeutic decisions are often made on the ability to distinguish infection from colonization. The concept of carriage (25) or colonization (24) refers to the presence of the fungus or its DNA in the absence of clinical pneumonia. Since most of the BAL procedures here were performed for investigating fever and/or pneumonia, the detection of *P. jirovecii* DNA cannot be regarded as simple carriage. For some authors, cases corresponding to positive qPCR and negative microscopy warrants specific therapy when immunosuppression is ongoing (27). Our results suggest that this statement may need to be discussed according to the underlying disease keeping in mind the limits of our present study. Because of the retrospective design of the study, the qPCR results were not available to guide clinical decision-making, which was done based on IFA results alone. Furthermore, prescription and observance of anti-PcP prophylaxis, doses of steroids, or concomitant immunosuppressive drugs introduced after the BAL results could not be analyzed in the absence of a common follow-up. Likewise, the cause of death in this immunocompromised population could not be determined retrospectively because of lack of pre-study defined criteria. For HIV-positive patients, PcP risk largely depends on the CD4+ T cell count and both treatment and prophylaxis are well established (27). For hematological patients and SOT recipients, the presence of *P. jirovecii* DNA did not seem to impact the overall survival at one year. However, co-trimoxazole at a prophylactic dose can be resumed whatever the qPCR result in
patients with hematological malignancy and SOT recipients (9, 13). In contrast, for patients receiving high doses of steroids for inflammatory diseases, the IFA-negative/qPCR-positive patients had a worse overall one-year survival than the qPCR-negative ones. There is currently no recommendation for anti-PcP prophylaxis in autoimmune diseases with the exception of Wegener granulomatosis (13). Despite this, some authors have encouraged anti-PcP prophylaxis in patients receiving immunosuppressive therapy for rheumatoid arthritis (23). The opposing argument is that some patients would probably recover without specific therapy, as observed in our study.

Given its high reproducibility and with the emergence of commercially available tests (14), there is no doubt that qPCR has the potential to replace microscopy with the help of the necessary quality control panels. More importantly, qPCR has the potential to give reliable quantification and can address the issue of correlation between tissue burden and outcome (16). Then, qPCR-based therapeutic strategies could be evaluated in homogenous groups of patients keeping in mind the potential toxicity of anti-PcP treatments. Therefore, operational thresholds should be established from prospective studies.

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Potential conflicts of interest. All authors: no conflict.
References


Legend to Figure

Figure 1: One-year survival of patients with hematological malignancy (A) and of patients receiving high dose steroid therapy (B) according to the qPCR results (patients with microscopy-positive BAL sample and thus treated with full dose co-trimoxazole were excluded). A: no statistically difference was noted between the qPCR-positive and qPCR-negative patients (p=0.26). B: a statistical difference was observed between the qPCR-positive and qPCR-negative patients (p<10^-3).
Table 1: Overview of data obtained from three separate runs of standard curves with serial
dilutions of a plasmid clone containing the target for *P. jirovecii* DNA detection. Each
dilution was analysed in duplicate. The coefficients of variation (CV) show good
reproducibility.

<table>
<thead>
<tr>
<th>Plasmid concentration (copy/µl)</th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>10⁷</td>
<td>6.88</td>
<td>6.53</td>
<td>7.09</td>
<td>0.24</td>
<td>3.5%</td>
</tr>
<tr>
<td>10⁸</td>
<td>10.41</td>
<td>10.22</td>
<td>10.51</td>
<td>0.11</td>
<td>1.0%</td>
</tr>
<tr>
<td>10⁷</td>
<td>14.16</td>
<td>13.74</td>
<td>14.47</td>
<td>0.27</td>
<td>1.9%</td>
</tr>
<tr>
<td>10⁶</td>
<td>17.72</td>
<td>17.26</td>
<td>17.96</td>
<td>0.32</td>
<td>1.8%</td>
</tr>
<tr>
<td>10⁵</td>
<td>21.03</td>
<td>20.85</td>
<td>21.14</td>
<td>0.12</td>
<td>0.6%</td>
</tr>
<tr>
<td>10⁴</td>
<td>24.54</td>
<td>24.19</td>
<td>24.73</td>
<td>0.24</td>
<td>1.0%</td>
</tr>
<tr>
<td>10³</td>
<td>28.21</td>
<td>27.72</td>
<td>28.50</td>
<td>0.32</td>
<td>1.1%</td>
</tr>
<tr>
<td>10²</td>
<td>31.97</td>
<td>31.32</td>
<td>32.46</td>
<td>0.48</td>
<td>1.5%</td>
</tr>
<tr>
<td>10</td>
<td>34.91</td>
<td>34.02</td>
<td>35.75</td>
<td>0.68</td>
<td>1.9%</td>
</tr>
<tr>
<td>1</td>
<td>39.32</td>
<td>38.98</td>
<td>39.57</td>
<td>0.20</td>
<td>0.5%</td>
</tr>
</tbody>
</table>
Table 2: Main characteristics of the 353 BAL fluids of this study according to the qPCR results.

<table>
<thead>
<tr>
<th></th>
<th>IFA-positive</th>
<th>IFA-negative</th>
<th>IFA-negative</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>qPCR-positive</td>
<td>qPCR-positive</td>
<td>qPCR-negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=17 (4.8%)</td>
<td>n=63 (17.8%)</td>
<td>n=273 (77.3%)</td>
<td></td>
</tr>
<tr>
<td>Mean recovered volume (SD)</td>
<td>78.5 (18.2)</td>
<td>61.8 (22.2)</td>
<td>66.2 (25.0)</td>
<td>0.058</td>
</tr>
<tr>
<td>Mean log10 copy number (SD)</td>
<td>4.2 (1.2)</td>
<td>1.1 (1.1)</td>
<td>-</td>
<td>&lt;10^-4</td>
</tr>
<tr>
<td>No. with diffuse radiological patterns (%)</td>
<td>15 (88.2)</td>
<td>27 (42.9)</td>
<td>94 (34.4)</td>
<td>&lt;10^-4</td>
</tr>
<tr>
<td>No. with anti-PcP prophylaxis (%)</td>
<td>1 (5.9)</td>
<td>2 (3)</td>
<td>51 (18.7)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

SD: standard deviation
Table 3: Detailed characteristics according to immunofluorescence (IFA) and quantitative PCR (qPCR) results of the 353 BAL fluids studied

<table>
<thead>
<tr>
<th>BAL Patients (%)</th>
<th>IFA positive / qPCR positive</th>
<th>IFA negative / qPCR positive</th>
<th>IFA negative / qPCR negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of samples (%)</td>
<td>Total copy no. (SD)</td>
<td>Total copy no. (SD)</td>
</tr>
<tr>
<td>No concerned</td>
<td>353</td>
<td>287</td>
<td>17</td>
</tr>
<tr>
<td>HIV+ve patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥200 CD4</td>
<td>46 (13)</td>
<td>39 (14)</td>
<td>8 (47)</td>
</tr>
<tr>
<td>&lt;200 CD4</td>
<td>30 (12)</td>
<td>13 (39)</td>
<td>0 (8)</td>
</tr>
<tr>
<td>Hematology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute leukemia</td>
<td>147 (42)</td>
<td>111 (39)</td>
<td>6 (35)</td>
</tr>
<tr>
<td>Chr. lymph. dis.</td>
<td>74 (17)</td>
<td>62 (111)</td>
<td>3.6</td>
</tr>
<tr>
<td>SOT recipients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>heart</td>
<td>57 (16)</td>
<td>47 (16)</td>
<td>2 (12)</td>
</tr>
<tr>
<td>liver</td>
<td>17 (5)</td>
<td>13 (47)</td>
<td>0.2</td>
</tr>
<tr>
<td>kidney</td>
<td>29.57</td>
<td>23.47</td>
<td>0.2</td>
</tr>
<tr>
<td>Syst. Infl. Dis.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute leukemia</td>
<td>53 (15)</td>
<td>47 (16)</td>
<td>1 (6)</td>
</tr>
<tr>
<td>Others</td>
<td>32 (9)</td>
<td>27 (9)</td>
<td>0</td>
</tr>
</tbody>
</table>

a Chr. lymph. dis = chronic lymphoproliferative disorders; SOT = Solid Organ Transplant; Syst. Infl. Dis. = systemic inflammatory diseases
b in log copy number/µl; SD = standard deviation
c Diffuse radiological lesions
d PcP Proph = PcP prophylaxis