Quantitative Real-Time PCR Compared with pp65 Antigen Detection for Cytomegalovirus (CMV) in 1122 Blood Specimens from 77 Patients after Allogeneic Stem Cell Transplantation: Which Test Better Predicts CMV Disease Development? Andreas Nitsche, Olivia Oswald, Nina Steuer, Johannes Schetelig, Aleksandar Radonić, Stefanie Thulke, and Wolfgang Siegert (Medizinische Klinik II m.S. Onkologie und Hämatologie, Charité, Humboldt Universität, 10117 Berlin, Germany; * current address: Robert Koch-Institut, Zentrum für Biologische Sicherheit 1, Norddefer 20, 13353 Berlin, Germany; † address correspondence to this author at: Medizinische Klinik II m.S. Onkologie und Hämatologie, Charité–Campus Charité Mitte, Schumannstrasse 20-21, 10117 Berlin, Germany; fax 49-30-450-513952, e-mail Wolfgang.Siegert@charite.de)

Cytomegalovirus (CMV) can compromise the life of patients who are immunosuppressed after organ transplantation or who have acquired immunodeficiency syndrome. CMV infections or reactivations can be successfully treated, provided treatment is instituted early in the course of developing disease. Current assays to detect CMV pp65 antigen in peripheral blood leukocytes or CMV DNA by qualitative PCR of leukocytes or plasma can be slow and tedious (1–3). Antigen detection is limited by the availability of sufficient white blood cell numbers and is impossible to perform on leukopenic patients. In addition, blood for antigen detection cannot be stored for long periods because white blood cells need to be intact for staining procedures (4, 5). PCR techniques detect relatively stable double-stranded DNA present in the plasma or whole blood regardless of storage conditions or cell integrity, but qualitative PCR assays are not useful for follow-up studies of patients, and they do not allow the determination of viral load, which is useful for prognosis.

In this study we compared quantitative real-time PCR (6, 7) with conventional pp65 antigen staining among 77 patients (1122 blood samples) surviving more than 30 days after stem cell transplantation (see Table 1 in the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol49/issue10/). In a retrospective study, results of the two assays were compared, and their sensitivity and specificity for predicting CMV disease were calculated.

All patients were transplanted in the Virchow Klinikum (Berlin, Germany) between January 1998 and March 2001 (see Table 1 in the online Data Supplement). Five patients developed CMV organ disease, which included pneumonitis and hepatitis (n = 1), hepatitis (n = 1), enteritis (n = 2), and retinitis (n = 1). Seven additional patients developed thrombo- and leukopenia not explained by other conditions. pp65 antigen detection was performed as described previously (3). Leukocytes were prepared from the same blood sample as plasma DNA according to methods reported previously (2, 3). Specimens were scored positive when 1 or more leukocytes per 10 000 cells were stained. Quantitative real-time PCR was performed, and the results analyzed as described previously (6, 7). Comparisons between groups were calculated with the χ² test or the Mann–Whitney U-test. Differences were considered significant when the P value was <0.05. Correlation was calculated with the Spearman rank test.

Each blood sample was studied masked by real-time PCR and pp65 antigen detection until results of the two assays were available. The median number of samples studied was 15 (range, 3–41) per patient. Forty-three of 1122 (3.8%) samples were positive in both the PCR and pp65 antigen assay, and 944 of 1122 (84.1%) were negative in both tests (see Table 2 in the online Data Supplement). PCR and pp65 antigen assay results were discordant for 135 of 1122 (12.0%) of samples. In summary, PCR detected 79 of 1122 (7%) positive samples that were scored negative in the pp65 reaction (P <0.0001).

The quantitative correlation between PCR and antigen detection is shown in Fig. 1. PCR-negative samples had a median of 1 positive cell (range, 1–30 cells), whereas PCR-positive samples had at a median of 1 positive cell (range, 1–300 cells). Quantitative real-time PCR in cell samples without and with pp65-positive cells (A). The observed difference was statistically significant (P = 0.0007). (B), CMV loads in cell samples without or with 1–10 or 11–200 pp65-positive cells per 10 000 leukocytes. The observed differences were statistically significant (P = 0.0005). Results for patients with diagnosed CMV disease are indicated by ⊙.
negative pp65 antigen detection had a median of 4134 genome-equivalents (GE)/mL (range, 2011–439 500 GE/mL), samples with 1–10 pp65-positive cells had a median of 6930 GE/mL (range, 2573–397 200 GE/mL), and samples with 11–200 antigen-positive cells had a median of 18 060 GE/mL (range, 3052–497 700 GE/mL; Fig. 1B). These differences were statistically significant (P = 0.0005).

The PCR and pp65 results for individual patients were both positive for 19 of 77 (25%) patients, and 34 of 77 (44%) were negative in both tests. Twenty-two (29%) patients were negative in the pp65 reaction but positive in PCR, and 2 (2.6%) were negative in PCR but positive in the pp65 reaction. In summary, an additional 26% of patients were determined to be CMV positive by PCR than by pp65 antigen reaction: 41 of 77 (53%) vs 21 of 77 (27%), respectively (P < 0.0001; see Table 3 in the online Data Supplement).

To compare the performance of PCR vs pp65 antigen assay, we used the time taken for each test to become positive after transplantation. PCR was first in 33 of 77 (43%) patients, whereas the pp65 assay was first in 9 of 77 (12%) patients. The median time for PCR to become positive was 35 days (range, 0–252 days) compared with 49 days (range, 6–75 days) for the pp65 reaction (P < 0.0001).

Patients with organ disease caused by CMV infection had a significantly higher virus load (median, 48 452 GE/mL; range, 8060–497 743 GE/mL) compared with patients without disease (median, 7022 days; range, 2023–397 228 days; P = 0.012). Similarly, the number of pp65-positive cells was significantly higher in patients with organ disease (median, 66 positive cells; range, 2–200 cells) compared with patients without disease (median, 3 cells; range, 1–30 cells; P = 0.025).

Although a correlation between real-time PCR and pp65 antigen detection was reported in previous studies (8), in this study, we found no clear correlation between viral DNA load in plasma and the number of pp65-positive leukocytes [Spearman coefficient (ρ) = 0.53]. This observation is in contrast to recent reports of better correlations, which were, however, based on comparatively low patient and sample numbers and may therefore incompletely reflect clinical reality (9–11). Looking at individual samples, it is in fact not surprising that antigen detection and PCR have only a moderate correlation because different target molecules are detected in different blood compartments, i.e., pp65 antigen in leukocytes and CMV DNA in plasma. We have previously found that the two molecules can follow differing time courses (6).

We have also found that the high sensitivity of real-time PCR assays permits the detection of as few as 10 GE/assay, corresponding to 2000 GE/mL of plasma (7). High sensitivity is an important aspect. As a consequence of the extraordinarily high sensitivity provided by real-time PCR, we detected CMV in 6% more samples and 26% more patients compared with the antigen assay. CMV DNA has been reported to be detectable in patients who developed CMV disease but did not become positive in the pp65 assay (12). In addition, PCR becomes positive ~2 weeks earlier than antigen detection. Other studies demonstrated that CMV could be detected even earlier and at higher quantities when leukocytes were used instead of plasma (13). Such a sensitive test may be of help in situations in which it is important to detect low amounts of virus early in the course of patient care. However, as shown in previous reports, PCR appears to be too sensitive to reliably identify patients at risk to develop CMV disease after stem cell transplantation (8).

A positive PCR result >2000 GE/mL has a much lower specificity and positive predictive value (PPV) for CMV disease than qualitative pp65 detection. However, assuming a threshold of >7500 GE/mL of plasma, the specificity and PPV of PCR are much closer to those of the pp65 antigen detection (see Table 4 in the online Data Supplement). A similar cutoff value of 7900 GE/mL of plasma was postulated previously for renal transplant patients as determined by the Roche Amplicor CMV DNA assay (14). Another approach to the prediction of cutoff values is the determination of the area under curve when plotting viral load over time (15). This method incorporates the knowledge that a long-lasting low viremia may produce the same clinical complications as a short-lived high viremia.

To address which assay is preferable, one must predetermine the purpose for which the assay is required: sensitive detection and monitoring of CMV infection or identification of patients at risk of CMV disease. Antigen detection assays that analyze 10 000 leukocytes have moderate analytical sensitivity, thereby exhibiting moderate PPV and specificity for the prediction of CMV disease in patients after stem cell transplantation. The sensitivity of real-time PCR depends on the assay conditions. PCR tests with high sensitivity have a low PPV and specificity, and threshold values need to be evaluated under clinically relevant conditions to obtain an appropriate specificity and PPV as close to 100% as possible to avoid unnecessary CMV treatment. In addition to the question of sensitivity, the availability of patient specimens may influence the choice of a test. pp65 antigen detection requires leukocytes from freshly collected blood, which cannot be stored or frozen. In contrast, real-time PCR can be performed with small volumes of plasma, which may be stored frozen for long periods without loss of DNA integrity.

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References

YKL-40 as a Marker of Joint Involvement in Inflammatory Bowel Disease, Daniela Bernardi,† Marta Podswiadek,‡ Martina Zaninotto,† Leonardo Punzi,‡ and Mario Plebani†*,††

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Rheumatic symptoms are the most common extra-intestinal manifestations of inflammatory bowel diseases (IBD). Both ulcerative colitis and Crohn disease (1, 2) can be complicated by seronegative spondyloarthropathies, including two principal patterns of arthritis: spondylitis and peripheral arthritis. Spondyloarthropathies resembling idiopathic ankylosing spondylitis occur in 10% of patients with ulcerative colitis and, less frequently, in those with Crohn disease; peripheral, often asymptomatic, arthropathies occur in 5–20% of IBD patients. Unlike arthropathy limited to five or fewer joints, polyarthropathy and spondyloarthropathies do not reflect the activity of the underlying IBD.

The “gold standard” for assessing joint damage remains the plain radiograph, which images only the bone and allows reliable detection of changes within a time span of at least 12 months. Importantly, all such techniques image only damage that has already occurred. Even after repeated investigations, they are of limited use in informing the clinician of continuing or future damage.

Biochemical markers may provide a valuable tool for the frequent quantitative measurements required for the diagnosis and monitoring of joint disease. Highly sensitive C-reactive protein (CRP) assays provide information on the inflammatory process (3) but are poor markers for joint disease in IBD. Other markers for joint disease have been proposed (4) that are related to structures within joints.

The concentrations of YKL-40 in synovial and synovial fluid are closely correlated in patients with joint disease (5), suggesting that most of the protein found in serum may be produced within the joint (6, 7). YKL-40 is produced not only by chondrocytes and synovial cells but also by macrophages, neutrophils, cancer cells, endothelial cells, smooth muscle cells in blood vessels, and by cells (probably hepatic stellate cells) in the fibrotic liver (8–13). The protein is a growth factor for fibroblasts, chondrocytes, synovial cells, and endothelial cells (12, 14, 15) and appears to be a nonspecific marker of inflammation, tissue remodeling, or ongoing fibrosis (16, 17). Its value in detecting joint damage is still under evaluation.

In the present study, we evaluated serum YKL-40 as a possible marker for both peripheral and axial arthropathies in patients with IBD, and we assessed its biological variation and compared it with that reported for CRP and serum amyloid A (SAA) (18, 19).

We studied 171 patients (median age, 47 years; range, 21–79 years), of whom 29 had psoriatic arthritis (PsA; cutaneous psoriasis and arthritis and tenosynovitis and/or enthesopathy), 66 had IBD (36 with Crohn disease and 30 with ulcerative colitis), and 76 had joint involvement in IBD (IBD; 44 with Crohn disease and 32 with ulcerative colitis) consecutively admitted to the Rheumatology Department of the University-Hospital of Padua between January 7 and December 20, 2002.

The diagnosis of IBD was based on clinical signs and symptoms, macro- and microscopic findings at endoscopy, and radiologic imaging. Patients were classified as active IBD (26%, of whom 4% had severe active disease) and nonactive IBD according to the international criteria for remission of IBD [for Crohn disease, a Crohn Disease Activity Index <150; for ulcerative colitis, a Powell-Tuck score <2 (20, 21)]. The arthropathies consisted of seronegative spondyloarthritis, with a clinical spectrum that included peripheral and axial involvement or isolated enthesopathy. Spondyloarthritis, diagnosed on the