the ligand-bearing cell, has also been reported [5]. In RA patients, high levels of sCD30 have been reported in blood and synovial fluid, and CD30+ T lymphocytes are present in significant numbers in the latter [6]. CD30+ T cells have been suggested to be involved in the anti-inflammatory response in early RA, possibly through association with IL-4 and IL-10 production [7]. However, the role of these cells in long-standing RA is probably different owing to a more complex cytokine network later in the disease process.

BV is a novel CD30-directed ADC approved for the treatment of relapsed Hodgkin’s and systemic anaplastic large cell lymphomas [8]. The conjugation of a CD30-specific monoclonal antibody to a potent microtubule-disrupting agent [monomethyl auristatin E (MMAE)] via a stable linker allows the targeted delivery of high doses of the cytotoxic drug to CD30-expressing cells, leading to high therapeutic efficacy [8]. Besides targeting CD30+ lymphocytes through direct binding, an important mechanism of action of BV may be the bystander effect of MMAE, which diffuses out of the CD30+ lymphocytes into their microenvironment [8]. As such, the efficacy of BV against RA could potentially be multifactorial: BV could directly alter the CD30/L interaction, besides exerting cytotoxic effects on CD30+ T cells, as well as surrounding inflammatory cells in the synovial milieu. As with other microtubule toxins such as taxanes, vinca alkaloids, epothilones, eribulin and the novel human epidermal growth factor receptor 2-targeted ADC T-DM1, potentially reversible peripheral neuropathy has been the principal safety concern in clinical trials of BV [8]. Our observations are intriguing and suggest activity of BV in RA that should be explored further in clinical trials. Besides potentially providing an important new option to patients with the disease, this may help further elucidate the pathophysiology of RA.

Rheumatology key message

● Brentuximab vedotin, a CD30-directed antibody-drug conjugate, may represent a novel targeted therapy for RA.

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Anticoagulating synovial fluid samples in septic arthritis

Sirs, Arthrocentesis techniques for SF analysis and culture in suspected septic arthritis must be re-evaluated. Gram-stain microscopy of SF can rapidly confirm the diagnosis, but previous studies have suggested a false negative rate of 25–50% [1–4], with some authors reporting rates of up to 78% [5]. Consequently, negative Gram-stain microscopy rarely influences management, but has the potential to delay treatment in a small number of cases. Treatment is usually commenced irrespective of the result and is almost always under way before culture results are available. Based on these figures, Gram-stain microscopy as used at present has limited clinical value in the diagnosis of septic arthritis.

Coagulation of SF before it reaches the lab may be to blame for the test’s low sensitivity. Although SF under
normal circumstances contains no clotting factors, any inflammatory arthropathy will increase local vascular permeability. This causes clotting factors to leak into the joint cavity [6], resulting in coagulation of the fluid in the container before it has reached the lab for analysis [6]. The resulting gelatinous fluid is extremely difficult to prepare adequately for microscopy [7]. Preventing coagulation of the SF sample allows more effective centrifuging, leading to preparation of a more homogeneous slide for microscopy. It follows that this intervention could improve the diagnostic value, and one suggestion for improvement is the use of heparinized containers to transport the SF sample from the bedside to the laboratory [5, 6].

In order to investigate the use of heparinized containers, we performed a retrospective study examining cases of septic arthritis that passed through our laboratory between 1998 and 2010. Cases were included if they cultured positive for septic arthritis. Culture-negative samples were excluded. All of the samples were collected in sterile lithium heparin containers, which anticoagulated the sample. All cases examined had the diagnosis of septic arthritis made after positive isolation of the organism in SF broth culture, and all cases included had this diagnosis confirmed clinically. Once this database had been created, the corresponding Gram-stain reports were examined. A negative Gram stain was considered a false negative, while a positive Gram stain was considered a true positive.

A total of 602 cases of culture-positive septic arthritis met our inclusion criteria between 1998 and 2010. Diagnosis of septic arthritis was confirmed clinically in all cases. Gram-stain microscopy was negative in 34 cases of septic arthritis, giving a false-negative rate of 5.6% and a sensitivity value of 94%.

Heparin may well be the ideal anticoagulant for the job. Its use in preventing coagulation of SF was first proposed in 1998, [8] and it was recently advocated in a review by Denton [6]. Heparin does not interfere with endogenous crystals, permitting polarized light and Gram-stain microscopy of the same sample. Moreover, heparin does not crystallize and thus will not produce false-positive results for crystal analysis [6]. To our knowledge, heparin has no effect on the growth of microorganisms in culture, however, we must emphasize the importance of using sterilized heparin containers in order to avoid possible bacterial contamination of samples, which may lead to false positives.

This is the largest study investigating the efficacy of Gram-stain microscopy in the literature. All our samples were collected in sterile heparinized containers. There are two possible limitations to our study. First, as no direct comparison has been made, we hypothesize that anticoagulation of our samples is directly responsible for this vastly increased sensitivity. It could be that the use of heparinized containers selects out samples with particularly fastidious organisms, which would directly result in an improved false-negative rate. We are unable to confirm or refute this. Second, due to retrospective identification of patients with diagnosed septic arthritis during the data collection stage, we were unable to calculate the false-positive rate for this cohort. Further data analysis of culture-negative cases revealed a separate subcohort with 82 cases of positive Gram-stain results but negative cultures, which could be interpreted as false-positive results. These patients were still diagnosed and treated for septic arthritis, however, and so interpretation of this statistic is problematic. Our cohort contained no true negatives, so it was not possible to calculate the false-positive rate. This should certainly be considered in future studies performing full statistical analysis on Gram-stain microscopy.

We report a sensitivity of 94% of Gram-stain microscopy, far higher than previously reported. We conclude that Gram-stain microscopy is still a valid investigation in the diagnosis of septic arthritis, but samples should be collected in sterile heparinized containers to increase the diagnostic yield.

Rheumatology key message

- Anticoagulating synovial fluid samples may increase the diagnostic yield of Gram-stain microscopy in septic arthritis.

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Differential methylation within the major histocompatibility complex region in rheumatoid arthritis: a replication study

Sir, Within a single decade the research on complex diseases such as RA has changed from hypothesis-driven genetic studies to discovery-driven genome-wide association studies, and presently the field is moving further to the evaluation of dysfunctions in multiple systems and changes at different levels, such as epigenetics, transcriptomics, proteomics and metabolomics. A key issue in discovery-driven approaches is the replication of findings. For the genome-wide SNP studies, the risk of false-positive findings due to multiple testing was dealt with by the requirement to replicate findings in independent datasets. For epigenetic studies, the design, analysis and interpretation is significantly more complex, and although the need for replication is well established, criteria remain to be developed [1].

Changes in DNA methylation presumably contribute to RA development [2]. Few genome-wide methylation studies have been performed thus far. Two studies compared fibroblast-like synoviocytes of RA patients and OA controls. Both studies comprised six RA and six OA samples [3, 4] and observed several differentially methylated loci. Different loci were found in these studies and validation of the results in independent populations was not included. The largest epigenome-wide study in RA was published recently by Liu et al. [5]. Using peripheral blood samples of 354 Swedish RA patients and 335 healthy controls as the identification set and monocytes of 12 patients and 12 controls as the validation set, they reported on 10 differentially methylated positions (DMPs) within and outside the MHC region at chromosome 6 in RA. The authors also used methodology to address confounding influences on DNA methylation from cell heterogeneity and the consequences of the disease itself [5]. Because of the relevance of independent replication, we aimed to evaluate the methylation levels at these 10 positions in peripheral blood mononuclear cells (PBMCs) of RA patients and healthy controls in two independent datasets of different continents.

The first replication dataset comprised PBMCs from 46 European RA patients (mean age 55.6 years, 63.0% female, 45.5% ACPA positive) and 15 healthy controls [6]. The PBMCs were collected at time of diagnosis (median symptom duration until diagnosis 4.7 months [interquartile range (IQR) 2.2–7.1]), reducing the risk that methylation was influenced by effects of long-standing inflammation or treatment. The second replication dataset included PBMCs of 21 North American RA patients (mean age 53.1 years, 53.1% female) and 12 healthy controls. Seven patients were untreated at the time of sample collection and 14 were treated with biologics (n = 8), and/or MTX (n = 7) and/or corticosteroids (n = 4). All subjects in both datasets were white. Epigenome-wide methylation assessment was done using the 450K methylation array (Illumina, San Diego, CA, USA). Methylation analysis of the European PBMC samples was initially done with the aim of identifying DMPs associating with DMARD-free sustained remission in RA; no epigenome-wide significant results were obtained here (data not shown). To evaluate methylation differences between the RA patients and healthy controls in the European dataset, methylation levels of the positions reported as differentially methylated by Liu et al. [5] were extracted. Methylation of the North American samples was obtained similarly, using the Illumina 450K methylation array. All analyses were adjusted for age and gender. All patients gave informed consent and approval was obtained from the medical ethics committee of the Leiden University Medical Center and the institutional review boards of the University of California, San Diego and BioMed (San Diego, CA, USA).

Fig. 1 presents the results; for direct comparisons, the methylation differences in cases vs controls as observed by Liu et al. [5] are also depicted. Although the methylation changes reported by Liu et al. were relatively small (1.2–6.6% difference in methylation), differences in methylation in the same direction were seen in both replication datasets for several positions. We first evaluated the DMPs within the MHC region. Cg21325723 was significantly associated with RA in the analysis combining both replication datasets (4.8% difference in methylation, P = 0.026). For cg16609995, cg19555708, cg19321684 and cg25949002 the effect sizes in our replication cohorts were comparable to the effect sizes observed by Liu et al. (Fig. 1), although statistical significance was not obtained. The fact that our number of samples was smaller than that of Liu et al. might have played a role. Liu et al. also reported on cg00462104 in GSTA2 outside the MHC region at chromosome 6; this association was not replicated in our two datasets (Fig. 1).

In conclusion, the present data support the finding that at least one DMP in the MHC region (cg21325723) is associated with RA, a region that also harbours the most important genetic risk factor for RA. A major limitation is that Liu et al. [5] used full blood and monocytes and we studied PBMCs. Four of the 10 DMPs identified by Liu et al. in full blood were also found to be significant in monocytes. Interestingly, cg21325723, which was differentially methylated in PBMCs in the present study, was one of these four DMPs [5]. Another issue is that the number of RA samples in our datasets (67 in total) was