How well does synovial fluid gram staining correlate with cultures in native joint infections?

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Abstract

To evaluate the sensitivity and specificity of Gram staining of synovial fluid aspirated from native joints suspected to be infected, we reviewed results of synovial fluid Gram stain and cultures. The sensitivity and specificity of the synovial Gram stain were then calculated. From the 1067 consecutive synovial fluid samples evaluated, 830 samples fulfilled the set criteria. From these 830 synovial fluid samples, organisms were detected by culture technique in only 100 samples; most of which were Gram-positive bacteria (78%). The other 22% comprised Gram-negative bacteria, Fungi and a mixture of growth. Of these, concomitant Gram stain test revealed sensitivity and specificity of 17.0% and 99.7% respectively. Our study demonstrates that the Gram stain technique has low sensitivity in detecting organisms in presumed native joint infections. Our findings demonstrate that the Gram stain test is an unreliable investigation in diagnosing native joint infections.

Introduction

Septic arthritis is relatively uncommon, however, the squeal of undiagnosed or inadequately treated cases can be devastating. Moreover, the incidence in native joints is reported to be rising.

A recent population study revealed a rise in the incidence of native joint septic arthritis in the United Kingdom from 5.5 per 100000 person-years in 1998 to 7.8 per 100000 person-years in 2013.[1]

The consequences of failed diagnosis or inadequate treatment could result in the mortality rate of up to 11.5%, and a morbidity rate of up to 31.6%, largely due to complications such as osteomyelitis, subcondral joint bone loss and dysfunction, as well as septicaemia.[2,3] This underscores the importance of appropriate diagnosis and prompt treatment.

Arthrocentesis for Gram staining (as part of microscopy) and culture analysis is a well established early investigative procedure and this needs to be carried out on an urgent bases and preferably prior to commencement of antibiotics whenever feasible.[4-6]

In some institutions, the initial clinical decision on treatment is still based on the results of Gram stain analysis. This is likely due to the relative rapidity of performing the test with the possibility of results being available in as soon as an hour. Cultures on the other hand will require at least 24 hrs to 48 hrs of processing time for the primary culture, and 3-7 days for the extended cultures.

The Gram stain test has been in use since 1883 when Hans Christian Gram, a Danish physician, observed differential staining of lung tissue samples following application of reagents, and thus serendipitously discovered the test.[7] The microorganisms first identified by the technique were Diplococcus pneumoniae and Bacillus pneumoniae, now better known as Streptococcus pneumoniae and Klebsiella pneumoniae, respectively.[7] That same year, Carl Friedlander mentioned the Gram staining in an article for the first time.

The following year, Hans Christian Gram himself published a more detailed article on the Gram staining technique.[8] The first diagnostic use of Gram stain was thought to have been by Roux for gonococcus in 1886.[8] Although the Gram stain has undergone a number of modifications over the years, the basic principles remains unchanged. The test allows the categorisation of microorganisms into either Gram positive or Gram negative based on the ability of their cell walls to retain the crystal violet dye during the decolorization process. Those that retain the crystal violet dye (and thus appears purple) are classed as Gram positive, while those that do not are classed Gram negative. The Gram negative microorganisms still do retain some of the safranin counter stain, and thus can be visualised as pink coloured cells.[9]

Thus, the determination of the presence or absence of organisms in a sample using the Gram stain test is based on the colour retention of the cells as described above. The microscopic structure of the cells seen as a result of the staining also allow further categorization into subgroups such as cocci, (which can appear as either clusters or chains) or bacilli which appear as rods.

Certain bacteria cannot be visualized by Gram stain, either due to the absence of cell wall (e.g. Mycoplasma species) or because their cell wall structure does not retain Gram stain reagents (eg, Chlamydia and Mycobacterium species).[9]

Gram stain is still frequently requested in many institutions as part of the initial investigation despite published studies on its low sensitivity.

The primary aim of this study is to evaluate the correlation of synovial fluid Gram staining and cultures in order to determine if the Gram stain test is indeed sensitive enough to be considered a necessary test.

Materials and Methods

This was a retrospective observational study in which we reviewed the reports of a cohort of synovial fluid samples retrieved from suspected infected joints and sent to our microbiology department for analysis. The results of the Gram staining microscopy (GSM) and culture analyses reviewed, were those performed between August 2015 and August 2017. Only samples from native joints were retrieved in this study. To enhance stringency of the study, samples were excluded if: (i) the results were reported by the laboratory as “possible

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contaminants" or "of doubtful significance"; (ii) patient details were duplicated; (iii) samples were sent specifically to investigate for tuberculosis (as this requires a different technique for detection); and (iv) GSM and concomitant culture reports were unavailable.

The knee was found to constitute 54% of the joints involved in patients who presented to the hospital with features suggestive of septic arthritis, during our study period. Others include: elbow (20%), hip (8%), ankle (7%), wrist (6%) and shoulder (5%).

A positive Gram stain microscopy (GSM) test was one that was reported as 'organisms seen' (which is usually accompanied by a description of the structure of the organism) and a negative test was one recorded as 'no organisms seen'.

For this study, synovial fluid culture was the gold standard technique for detecting infection.

The sensitivity and specificity of the Gram stain test were calculated, as were the positive and negative predictive values and the 95% confidence intervals (CIs) were for all samples. The statistical analysis was performed using the Medcalc statistical software.

The type and frequency of occurrence of the organisms cultured were additionally reviewed, as were any variation in the stage of culture at which the organisms were detected.

Results

A total of 1067 samples were reviewed and after application of the exclusion criteria already mentioned in the methods section, 237 samples were excluded, and the final number of samples evaluated was 830 samples. From 830 samples, culture analysis yielded organisms in 100 samples and of these, 78 samples contained exclusively Gram-positive bacteria, 17 contained exclusively Gram-negative bacteria, 3 contained fungi only and 2 samples contained a mixture of organisms. Of the 78 samples that contained exclusively Gram-positive bacteria, organisms were detected by Gram staining in only 17 samples. No organisms were detected by Gram staining in the rest of the aforementioned groups of samples.

In the 730 samples, which were culture negative, Gram staining detected organisms in 2 of the samples and these were considered as false positives. However, other possibilities of this occurrence include the presence of anaerobes, prior antibiotic therapy, or the presence of fastidious organism that cannot grow on routine media but yet detected on Gram staining.

Statistical analysis of the results revealed the synovial fluid Gram stain sensitivity and specificity as 17.0% (95% confidence interval: 10.2% to 25.8%) and 99.7% (95% confidence interval: 99.0% to 100%) respectively. The positive and negative predictive values were 89.5% (95% confidence interval: 66.6% to 97.3%) and 89.8% (95% confidence interval: 88.9% to 90.6%) respectively.

Discussion

The consequences of a missed diagnosis of septic arthritis can be devastating, as the infection requires prompt intervention. Moreover, an investigation with a low sensitivity can provide false negative results in the presence of true infection.

In our institution, the result of a Gram stain microscopy (GSM) is typically available within an hour and that of a culture is made available between 2 days – 7 days depending the culture stage reported. Our study reveals that the Gram stain has very

Table 1. Isolated pathogens from synovial fluid culture.

Class	Organism	Frequency of occurrence, %	% detection	
			At primary culture	At extended culture
Gram positive	*Staphylococcus aureus*	33.94	83.78	16.22
Gram positive	CNS	28.44	22.58	77.42
Gram negative	*Escherichia coli*	10.09	72.73	27.27
Gram positive	*Streptococcus* Group A	4.59	100	0
Gram positive	*Streptococcus* Group G	2.75	66.67	33.33
Gram positive	Corynebacterium	1.83	100	0
Gram positive	*Streptococcus* Pneumoniae	1.83	100	0
Gram negative	Pseudomonas	1.83	50	50
Gram negative	Pasteurella multocida	1.83	100	0
Gram negative	*Enterococcus* Faecalis	1.83	100	0
Gram positive	*Streptococcus* Group B	0.92	100	0
Gram positive	*Streptococcus* Group C	0.92	100	0
Gram positive	*Streptococcus* mitis/oralis	0.92	0	100
Gram positive	Bacillus	0.92	0	100
Gram positive	Anaerobes	0.92	0	100
Gram negative	Morganella Morganii	0.92	100	0
Gram negative	Proteus Species	0.92	0	100
Gram negative	Klebsiella	0.92	100	0
Fungi	Microspaeropsis	0.92	100	0
Fungi	Scedosporium	0.92	100	0
Gram negative	Campylobacter	0.92	0	100
Gram negative	Enterobacter	0.92	100	0

low sensitivity (17%) in detecting culture confirmed microorganisms, which is in keeping with many published studies. However, from our observation, majority of published studies appear to be on prosthetic, rather than native joints. This further underscores the importance of our study.

Oethinger *et al.* (2011) evaluated the sensitivity and specificity of Gram stains of macerated periprosthetic tissue; and the gold standard used for diagnosing infection was results of the microbiologic culture of the same fluid or tissue sample. They reported a final Gram stain sensitivity and specificity of 9% and 99% respectively in 390 specimens analysed.[10]

Zywiel *et al.* evaluated the sensitivity of Gram stain microscopy of intra-operative wound swabs acquired from 347 prosthetic knees.[11] In their study, the diagnosis of infection was based on the presence of one or more of following conditions: (i) two or more positive intra-operative or joint aspirate cultures with the same organism; (ii) histological evidence of an acute inflammatory response seen on frozen sections analysis of intra-operative samples; (iii) gross purulence; or (iv) a communicating sinus tract. They reported a Gram stain sensitivity of 7%, specificity of 99% and positive and negative predictive values of 92% and 57%, respectively. They recommend that Gram staining no longer be performed at the time of suspected periprosthetic knee arthroplasty infection.[11] More specifically for native joints, a recent study by Stirling *et al.* found a 78% false-negative rate of Gram stain test for the diagnosis of septic arthritis in their final cohort size of 143 positive synovial fluid culture results.[12]

Gram staining was performed on tissue and fluid samples obtained intraoperatively. They considered the joint arthroplasty to be infected if an organism could be isolated from cultures on solid media or if both the leukocyte count was >1,760 cells/μL and polymorphonuclear cell count was >73%, or if there was either a draining sinus tract or an abscess present. They considered the Gram stain test as positive if it can identify an organism, as well as if >5 polymorphonuclear cells were microscopically visualized in high-power field (which in our opinion, should have been more appropriately referred to as findings on microscopy and separate from findings of the Gram stain test). Using the aforementioned criteria to imply a positive Gram stain test, they reported the sensitivity of Gram stain as 43% and 64% for the total hip arthroplasties and total knee arthroplasties evaluated respectively, as well as a negative predictive values of 82% for both.[13]

Overall, the sensitivity of the Gram stain test reported in literature is thought to range from 0 to 64%.[10-15]

In our study, 22 different organisms were cultured from the synovial samples we reviewed; with the two most common causative organisms belonging to the *Staphylococcus aureus and* Coagulase negative *Staphylococci* (CNS) groups and both of which account for 62.4% of the cultured organisms Table 1. This is keeping with the frequency of organisms widely published in literature.[16,17]

Escherichia coli accounts for the 3rd most common organism found in our study.

With the culture technique, organisms are typically detected at either primary culture stage (i.e. at approximately 48 hours of sample processing) or at extended culture stage (which is usually within 3- 7 days of sampling). The Coagulase-Negative *Staphylococci* (CNS) group of organisms were mostly detected at extended culture stage, which could be due to its relatively low virulence as compared to *Staphylococcus aureus*, which is mostly detected at the primary culture stage Table 1. Synovial white cell count (WCC) is another test that can be performed during microscopy. However, this needs to be interpreted with caution as both infective and non-infective (but inflammatory) pathologies can result in high synovial WCC and distinguishing between both can challenging.[18] Furthermore, in addition to septic arthritis, Crystal arthropathy (*i.e.*, gout, pseudogout), Rheumatoid arthritis, juvenile idiopathic arthritis, Spondyloarthritis, Systemic lupus erythematosus and Lyme disease can all result in significantly raised (>2000 WCC per mm^3or > $2x10^9$ per L) synovial WCC.[19,20] Trampuz *et al.* quoted a synovial WCC figure of >1700 cells/μL and synovial fluid leukocyte differential of > 65% as having a sensitive of 94% and 97% respectively, for detecting infection in a total knee replacement in patients without underlying inflammatory joint disease and who were more than 6 months from primary prosthetic implantation.[21] Similar figures are often used as reference in units that routinely perform synovial white cell counts. In our unit, actual synovial white cell count (WCC) is not typically performed, alternatively the amount of synovial WCC seen are graded into 1+, 2+ or 3+. Our study has certain limitations- firstly; we only evaluated results of fluid samples and not tissue samples. This was because only synovial fluid analyses were performed in many of the native joints. Secondly, our study is retrospective and as such we could ascertain that all the patients were free of antibiotic use prior to arthrocentesis. Nevertheless, the impact of antibiotics would be expected to affect the results of both Gram stain and culture analyses so may likely have little impact on the correlation of Gram stain and culture.

Thirdly, we did not evaluate white cell count done as part of microscopy due to the reason already mentioned earlier in the discussion.

Conclusions

With such low sensitivity, the Gram stain test has poor correlation with conventional culture technique and is thus an unreliable in diagnosing joint infection. If microscopy is to be performed, a white cell count may be of more benefit but this has to be interpreted with the aforementioned caveats.

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