Hemolysis as a Clinical Marker for *Propionibacterium acnes* Orthopedic Infection

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Abstract

Determining if a *Propionibacterium acnes* culture is a true infection or a contaminant remains a challenge.

We conducted a study to distinguish between a true infection and a contaminated culture based on the *P acnes* hemolytic phenotype and clinical presentation. All *P acnes* strains were from orthopedic patients who had undergone arthroplasty or non-arthroplasty shoulder procedures. Hemolysis was determined according to *P acnes* growth on brucella blood agar plates after 48 to 72 hours. Each patient record that corresponded to the obtained *P acnes* strains was retrospectively reviewed for clinical data. An orthopedic surgeon involved in the care of the patients, but blinded to the hemolytic status of the bacteria, classified these infections as *definite, likely,* or *unlikely*.

Of the 22 *P acnes* strains, 13 were hemolytic, and 9 were nonhemolytic. Of the 13 hemolytic strains, 10 were *definite* infections; only 3 of the 9 nonhemolytic strains were *definite* infections. Mean (SD) C-reactive protein level was significantly higher ($P = .03$) in the hemolytic group, 16 (11) mg/mL, than in the nonhemolytic group, 7.9 (10) mg/mL.

A hemolytic phenotype of *P acnes* may represent a more pathogenic strain of bacteria, and may be more likely to be found in patients with a definite infection with *P acnes* rather than a contaminated culture.

Total shoulder arthroplasty (TSA) is an effective treatment modality for glenohumeral osteoarthritis. As the rates for primary TSA increase, so does the number of revision procedures for periprosthetic infection. The diagnosis of periprosthetic infection can become a dilemma when the normal signs and symptoms of infection are absent, especially in those patients with subacute or delayed infections.[1]

Propionibacterium acnes is a Gram-positive, non–spore-forming bacillus that is classified as an anaerobe but that has aerotolerant properties as well.[1-3] *P acnes* traditionally has been categorized as a laboratory or handling contaminant and considered nonpathogenic, as it is one of the most abundant organisms found on routine skin cultures around the shoulder.[4,5] Despite previous thinking, *P acnes* has become an increasingly recognized pathogen in upper extremity surgery and often presents as a subacute or delayed infection.[1,6,7] It is speculated that *P acnes* colonizes the surgical site at time of prosthesis implantation and grows unrecognized by the body for an extended period through biofilm formation.[8-10] The usual clinical and laboratory indicators of delayed infection with this organism are often within normal limits, and cultures must be held 2 to 3 times longer than normal for successful growth of *P acnes*, making microbiological diagnosis challenging.[1,3]

Despite the increasing recognition of *P acnes* as a true pathogen, it still may be only a contaminant in certain clinical situations. Uncertainty about the reliability of initial culture findings with this organism may lead to multiple joint aspirations in the preoperative setting and add to patient discomfort and morbidity. Previous work has shown variability in the pathogenicity of *P acnes* strains, suggesting some strains may be more aggressive than others during a deep infection.[11,12] *P acnes* can create biofilms and induce hemolysis and is not routinely identified on Gram stain, making it a formidable pathogen to eradicate without revision surgery.[13-17] It is not known if certain phenotypic characteristics correlate with the clinical and laboratory findings in patients with positive *P acnes* cultures. It would be useful to have an easily identifiable characteristic of *P acnes* that could assist the clinician in identifying a positive culture as being a true infectious agent rather than a contaminant.

We conducted a study to find an easily identifiable phenotypic characteristic of *P acnes* in isolates obtained from orthopedic upper extremity surgery. We hypothesized that patients identified as having *P acnes* strains with a hemolytic phenotype on brucella blood agar would have a more aggressive infection based on preoperative blood work and clinical course as compared with patients with nonhemolytic strains.

Materials and Methods

The institutional review board of the State University of New York at Buffalo approved this study. Since September 2010, our microbiology laboratory has saved all clinical strains of

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P acnes that have been identified, regardless of source. We retrospectively reviewed the records of all orthopedic patients with positive *P acnes* cultures included in this microbiology database. All strains were from consecutive orthopedic patients who had positive cultures and had undergone arthroplasty (n = 10) or nonarthroplasty (n = 12) upper extremity procedures between September 2010 and March 2012. Culture specimens were collected from either joint aspiration fluid or intraoperative cultures taken for suspected infection. All patients with positive *P acnes* cultures identified after upper extremity orthopedic surgery were included in this study for clinical and laboratory evaluation. Patients were excluded from evaluation only when preoperative laboratory and clinical symptoms could not be identified.

Bacterial samples were streaked onto brucella blood agar plates using a flame-sterilized wire loop, and hemolysis was determined based on *P acnes* growth after 48 to 72 hours under anaerobic conditions at 37°C. Hemolysis was recorded as positive if clearance around the bacterial colonies was more than 2 mm (**Figure 1**). Patient records that corresponded to the obtained strains were retrospectively reviewed for presenting symptoms, preoperative and operative culture data, clinical course, C-reactive protein (CRP) level, erythrocyte sedimentation rate (ESR), and white blood cell (WBC) count at time of diagnosis of the infection and before antibiotic use. Using the culture data and clinical course, a fellowship-trained shoulder and elbow orthopedic surgeon involved in the care of the patients, but blinded to the hemolytic status of the bacteria, classified these infections as *definite*, *most likely*, or *unlikely*. Patients with *definite* infections had 2 or more positive *P acnes* cultures, signs of infection during revision surgery (cloudy fluid or purulence), intraoperative pathologic signs of acute inflammation (> 10 WBCs per high-powered field), or they presented with signs and symptoms of an overt infection (draining sinus, erythema). Patients with *unlikely* infections had an isolated, unexpected positive culture or low suspicion based on presenting clinical symptoms, or ultimately were not treated for a true infection with antibiotics. Patients with *likely* infections had 1 positive *P acnes* culture with higher clinical suspicion for infection based on elevated laboratory values and clinical presentation, but they did not have the overt signs of infection and were treated clinically as having a true infection.

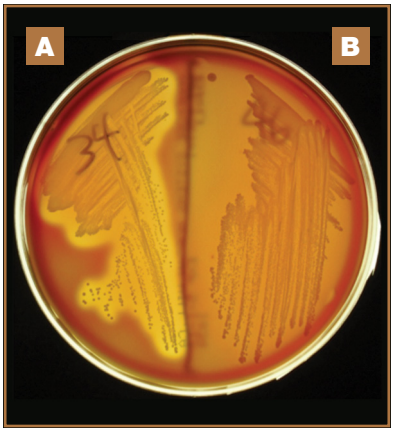

Figure 1. Hemolytic (A) and nonhemolytic (B) strains of *P acnes*.

CRP level, ESR, and WBC count were analyzed using nonparametric Mann-Whitney tests. $P < .05$ was considered statistically significant. Descriptive statistics were used to represent the patients grouped into *definite*, *most likely*, and *unlikely* infection categories. Sensitivity, specificity, negative predictive value, and positive predictive value were calculated for the ability of the hemolytic phenotype to predict true infection.

Results

The 12 men and 10 women in the study were evenly distributed between the hemolytic and nonhemolytic groups. Mean age was 58.3 years (range, 33 to 73 years) for the hemolytic group and 63.8 years (range, 40 to 80 years) for the nonhemolytic group. Of the 22 patients, 3 (2 hemolytic, 1 nonhemolytic) did not have presenting clinical symptoms, and 3 (2 hemolytic, 1 nonhemolytic) did not have documented presenting ESR and CRP records. All patients were included in the final infection classification. Patients without initial ESR and CRP data were not included in the laboratory analysis. Full clinical and laboratory data were available for review on the other patients.

A β-hemolytic phenotype was found in 13 of the 22 strains studied. Ten of the 13 hemolytic strains and 3 of the 9 nonhemolytic strains were *definite* infections. When the patients with *definite* and *likely* infections were included, all 13 hemolytic patients and 5 of the 9 nonhemolytic patients were identified (**Figure 2**). Sensitivity and specificity of the hemolytic phenotype in determining *definite* and *likely* infections from a contaminated culture were 72% and 100%, respectively. The hemolytic phenotype had a 100% positive predictive value but only a 44% negative predictive value. **Tables I and II** outline the patient and culture characteristics for the hemolytic and nonhemolytic strains of bacteria, respectively. All patients with documented clinical symptoms presented with symptomatic shoulder pain, regardless of hemolytic phenotype. Six of the 13 patients in the hemolytic group had overt signs of infection, which included erythema, swelling, and wound drainage, whereas only 1 patient in the nonhemolytic group did.

Mean (SD) CRP level (normal range, 1 to 5 mg/mL) was significantly higher ($P = .03$) in the hemolytic group, 16 (11) mg/mL, than in the nonhemolytic group, 7.9 (10) mg/mL (**Figure 3**). Mean (SD) ESR (normal range, 0 to 15 mm/h) tended to be higher in the hemolytic strains, 17 (14) mm/h, than in the nonhemolytic strains, 11 (7.2) mm/h, but the difference was not statistically significant ($P = .23$). The finding for WBC counts (normal range, 4.8 to 10.8 × 10^9/L) was similar: mean (SD) of 9.3 (2.4) × 10^9/L for the hemolytic group and 6.9 (2.0) × 10^9/L for the nonhemolytic group ($P = .07$). The hemolytic and nonhemolytic groups had similar mean (SD) times for positive culture to result: 10 (4.8) days and 9.5 (3.8) days, respectively ($P = .97$).

Discussion

We conducted this study to correlate clinical outcomes and preoperative laboratory values with the hemolytic phenotype of clinical *P acnes* strains. To our knowledge, this is the first study to correlate a *P acnes* hemolytic phenotype with clinical outcomes and preoperative laboratory findings in upper extremity

orthopedic patients. Our data suggest that patients with cultures containing a hemolytic strain of *P acnes* may present with slightly higher preoperative laboratory values, and that these cultures likely represent a true infection rather than a contaminant or nonpathogenic colonizer. We noted the hemolytic phenotype to be 100% specific and 72% sensitive for identifying true infection from contaminated cultures. In the clinical laboratory setting, it is very easy to ascertain hemolysis status, which may provide additional information that clinicians can use to determine whether a positive *P acnes* culture represents a true pathogen or a contaminant.

Hemolysis, the result of enzyme release from bacteria causing lysis of red blood cells, is attributed to the pathogenic properties of the bacteria. The hemolytic phenotype of different

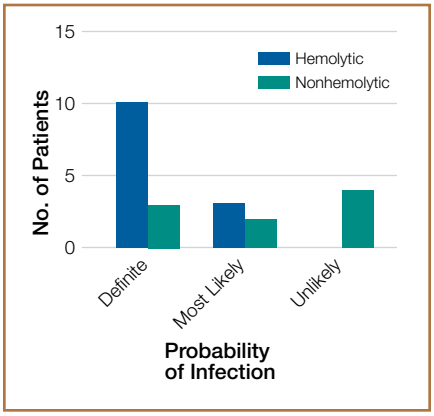

Figure 2. Probability of infection based on clinical course and culture data for patients with hemolytic and nonhemolytic strains of *P acnes*. Seventy-seven percent of group with hemolytic strains and 33% of group with nonhemolytic strains had *definite* infections. No patients in hemolytic group had *unlikely* infections.

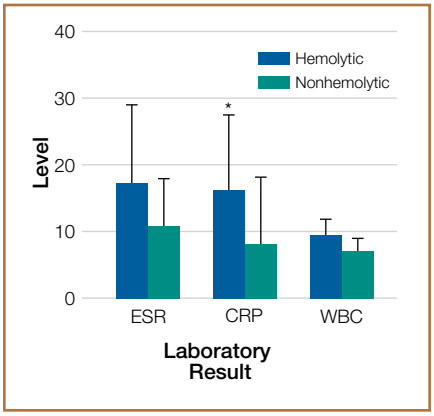

Figure 3. Mean preoperative laboratory results tended to be higher for patients with hemolytic strains of *P acnes* than for patients with nonhemolytic strains, but only mean (SD) C-reactive protein levels were statistically significantly higher: 16 (11) mg/mL for hemolytic group and 7.9 (10) mg/mL for nonhemolytic group ($P = .03$).

Abbreviations: CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; WBC, white blood cell count.

Table I. Laboratory and Culture Data for Patients With Hemolytic Strains of *Propionibacterium acnes*

Age, y	Sex	ESR, mm/h	CRP, mg/mL	WBC Count, × 10^9/L	Primary Operation	Presenting Symptoms	Positive *P acnes* Samples	Culture Type	Other Bacteria Cultured	Probability of Infection
33	M	12	22.9	11.5	Intramedullary nail, humerus	Surgical wound drainage	4/7	Intraoperative tissue and bone	None	Definite
34	F	NA	NA	12.5	ORIF, clavicle	Chronic draining wound	3/4	Intraoperative tissue and bone	None	Definite
63	F	27	21.7	7.2	Native shoulder	Nonspecific pain	1/1	Aspirate	None	Likely
65	M	9	6.7	8.8	Mini open RCR with arthroscopy	Wound swelling and erythema	5/5	Intraoperative tissue and bone	None	Definite
73	M	3	36.8	9.9	TSA	Generalized shoulder pain	3/8	Intraoperative tissue and bone	Coagulase-negative staphylococcus	Definite
57	M	35	21.2	12.5	Acromion and deltoid reconstruction	Wound swelling and erythema	4/5	Intraoperative tissue and bone	*Staphylococcus capitis*	Definite
66	M	5	6	7.9	Reverse TSA	Generalized shoulder pain	6/7	Intraoperative tissue and bone	None	Definite
70	F	35	3.7	6.5	Native shoulder	Generalized shoulder pain	1/1	Aspirate	None	Likely
61[a]	M	NA	NA	8.9	Shoulder hemiarthroplasty	Generalized shoulder pain	1/5	Intraoperative tissue and bone	None	Definite
63	F	10	3.5	7.8	Native shoulder	Generalized shoulder pain	2/2	Aspirate	None	Definite
61	F	7	6.9	5.6	TSA	NA	1/1	Aspirate	None	Likely
53	M	19	28.9	12.9	TSA	Wound swelling and erythema	5/5	Intraoperative tissue and bone	None	Definite
62	M	26	18.9	8.6	Arthroscopic RCR	Wound swelling and erythema	3/3	Intraoperative tissue and bone	None	Definite

Abbreviations: ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; WBC, white blood cell; NA, not applicable; ORIF, open reduction and internal fixation; RCR, rotator cuff repair; TSA, total shoulder arthroplasty.
[a]Patient had only 1 positive culture but more than 10 WBCs per high power field during surgery and was determined to be a definite infection.

strains of P *acnes* has been well described, and attributed to enzymes such as hyaluronidase, and chondroitin sulfatase.[15,18] We observed β-hemolysis, or complete hemolysis, in all strains of our bacteria—which is consistent with previous research and may suggest that these strains have pathogenic properties different from those of their nonhemolytic counterparts.[15] The general pathogenicity of P *acnes* has been suggested to be enhanced when it is a coinfectant with another bacterial species, which may be why at times it is found in polymicrobial cultures, and erroneously characterized as a contaminant in some clinical situations.[18,19]

The pathogenic differences between hemolytic and nonhemolytic strains may suggest a genomic cause for a more aggressive P *acnes* infection.[12] Sampedro and colleagues[20] evaluated P *acnes* phylotypes cultured from patients with failed orthopedic implants in an attempt to elucidate whether there is a genetic difference between pathogenic and nonpathogenic strains. The investigators did not find any correlation between the different phylotypes of those strains classified as the definite cause of the orthopedic implant failure versus those strains thought to be normal, nonpathogenic colonizing bacteria. Unfortunately, they did not evaluate the hemolytic phenotype of the P *acnes* strains in their study. Their work suggests a genomic difference may not be an ideal indicator differentiating pathogenic and nonpathogenic strains of P *acnes*, but further investigation may be indicated.

Butler-Wu and colleagues[3] retrospectively evaluated optimal recovery techniques for the diagnosis of a true periprosthetic joint infection with P *acnes*. The authors found that the highest percentage of P *acnes* strains recognized as true pathogens grew on brucella blood agar plates, but there was a high percentage of nonpathogenic strains that grew on this media as well. In our study, we noted growth on both CDC Anaerobe Blood Agar (Centers for Disease Control and Prevention) and brucella blood agar, but the most pronounced hemolysis was observed only with the brucella media. Both the CDC and brucella blood agars contain sheep blood, which suggests that mere presence of blood does not result in hemolysis, and that another difference between the 2 media contents may be facilitating this finding. Butler-Wu and colleagues[3] also noted P *acnes* growth in both aerobic and anaerobic environments, with 100% of infections identified with this method suggesting both types of cultures should be used. We used only anaerobic incubation in our study because we had known positive cultures of P *acnes*, and our main goal was to identify the hemolytic properties of our bacteria. It is well known that P *acnes* requires longer incubation for identification of growth, but Butler-Wu and colleagues[3] found increasing false-positive cultures of P *acnes* when growth was held past 13 days. Mean time to growth was about 10 days in both our hemolytic and nonhemolytic groups, which correlates with the identification of 90% of pathogenic strains of P *acnes*, and 60% of nonpathogenic P *acnes* strains in the study by Butler-Wu and colleagues.[3] All patients classified as *unlikely* to be infected in our study had cultures that resulted after 13 days of incubation, which supports the above findings and further suggests their correct classification as an *unlikely* infection.

Strengths of this study include the ability to analyze stored P *acnes* samples from consecutive patients with positive P *acnes* cultures from upper extremity orthopedic surgery, and the ability to classify patients into groups according to probability of infection using a blinded fellowship-trained upper extrem-

Table II. **Laboratory and Culture Data for Patients With Nonhemolytic Strains of *Propionibacterium acnes***

Age, y	Sex	ESR, mm/h	CRP, mg/mL	WBC Count, × 10^9/L	Primary Operation	Presenting Symptoms	Positive *P acnes* Samples	Culture Type	Other Bacteria Cultured	Probability of Infection
73	F	6.9	3.5	6.9	TSA	Generalized shoulder pain	1/3	Aspirate	None	Unlikely
65	M	9	3.5	5	Shoulder hemiarthroplasty	Generalized shoulder pain	1/2	Intraoperative tissue and bone	None	Likely
58	F	16	5.8	5.7	TSA	None	1/2	Intraoperative tissue and bone	None	Unlikely
77	F	5	5.9	7.5	Reverse TSA	Chronic shoulder pain	1/1	Aspirate	None	Definite
80	F	26	32.9	8.1	ORIF, proximal humerus	Generalized shoulder pain	1/2	Intraoperative tissue and bone	None	Unlikely
40	M	10	4.7	3.6	Acromioclavicular joint reconstruction	Wound swelling, erythema, drainage	2/2	Intraoperative tissue and bone	*Staphylococcus epidermidis*	Definite
66	F	NA	NA	NA	Reverse TSA	NA	1/1	Aspirate	None	Likely
53	M	4	3.5	9	ORIF, proximal humerus	Generalized shoulder pain	1/3	Intraoperative tissue and bone	None	Unlikely
63	M	8	3.5	9.1	Shoulder labral repair	Chronic shoulder pain	5/5	Intraoperative tissue and bone	MRSA in 1 culture	Definite

Abbreviations: ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; WBC, white blood cell; NA, not applicable; TSA, total shoulder arthroplasty; ORIF, open reduction and internal fixation; MRSA, methicillin-resistant *Staphylococcus aureus*.

ity surgeon who was involved in the care of these patients.

A major limitation of this study is lack of intraoperative histology findings. As only select cases had this information readily available for review, we could not use such findings as a major component in the placement of patients in their respective infectious categories. Another limitation is that we had only partial preoperative laboratory values for 3 patients—which had the potential to skew our results. Last, the case series was small. A larger series would increase the validity of our results. Upper extremity surgical infection rates are generally very low, and the incidence of cases with identified *P acnes* cultures is even lower. We intend to continue analyzing *P acnes* cultures as we identify them from our patient population. Despite these study limitations, we believe the variable hemolytic phenotype of *P acnes* we have described advances our knowledge of this organism and warrants further investigation.

Conclusion

Compared with patients with nonhemolytic strains of *P acnes*, patients with hemolytic strains of the bacteria were more likely to have *definite* infections with the organism, based on clinical course, and to present with significantly higher preoperative CRP levels. Hemolysis on brucella blood agar is an easily identifiable clinical laboratory finding that may represent a more pathogenic strain of *P acnes* bacteria, and care should be taken if a hemolytic strain of *P acnes* is considered a contaminant.

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This paper will be judged for the Resident Writer's Award.