Sensitivity and specificity analysis for somatic cell count (SCC) used to predict bacteriologically positive subclinical mastitis at calving in a dairy herd with low SCC

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Objective—Validate, by sensitivity and specificity analyses, use of somatic cell count (SCC) to predict bacteriologically positive subclinical mastitis in a California dairy herd with low SCC.

Design—Study of monthly dairy herd improvement SCC obtained from the immediate preceding lactation and individual cow composite milk sample microbiologic isolates collected at calving.

Animals—515 California dairy cows with SCC and culture data.

Procedure—Somatic cell count sensitivity and specificity analyses with combinations of SCC parameter and at various thresholds were done, using the bacterial isolates as the standard.

Results—Combination of SCC threshold and SCC parameters could not be developed that had sufficient sensitivity and specificity to be a useful predictor of cows that would calve with subclinical mastitis.

Clinical Implication—Under the conditions at this particular dairy, SCC could not be used as a basis of prediction of cows that would calve with bacteriologically positive subclinical mastitis or require selective nonlactating-cow antibiotic treatment. (*J Am Vet Med Assoc* 1996;208:1054–1057)

One of the current widely recommended control measures for mastitis in dairy herds is total herd nonlactating-cow antibiotic treatment at the end of lactation.[1,2] Nonlactating-cow treatment has been widely accepted on the basis of its economic benefit, compared with other methods, such as selective treatment.[2] This treatment has been documented to cure existing infections at the beginning of the nonlactating period and to reduce new infections in the early nonlactating period.[3,4] Producers and veterinarians have had increasing interest in selective nonlactating-cow treatment. Selective treatment might be possible if an accurate method for selecting cows for treatment was available. One possible method might be to select cows on the basis of somatic cell count (SCC).

If SCC were to be used to select cows for nonlactating-cow treatment, a critical threshold value would have to be established.[5] The threshold value would be used to identify infected cows to be treated at the beginning of the nonlactating period. Cows with SCC below the threshold value would not be treated.

For SCC to be useful, they would have to have suitable sensitivity and specificity values to produce a high degree of predicatablility for the test.[6] Earlier studies have determined sensitivities ranging from 70 to 90% and specificities ranging from 75 to 85% with a threshold SCC of 200 $\times$ 10^3/ml. Minor pathogens may alter these values.[6]

The purpose of the study reported here was to compare use of SCC with the standard of microbiologic analysis in a California dairy herd with low SCC that had a history of a low incidence of mastitis caused by gram-positive environmental pathogens.

Materials and Methods

During 1993, composite milk samples were aseptically collected from all clinically normal dairy cows in a California herd. Most milk samples were collected on day 3 after calving (range 2 to 5 days) by farm personnel who had been trained as part of a herd health program provided by their veterinarian (JPR). The milk samples were plated on blood agar, and bacterial growth was presumptively identified 18 to 24 hours later. Identification of the isolated bacteria by 1 of the authors (JPR) was confirmed by submission of over 200 samples to the Dairy Food Safety Laboratory at the College of Veterinary Medicine, University of California, Davis. Isolates were classified as no growth, *Streptococcus agalactiae, Staphylococcus aureus,* coliform, other *Streptococcus* spp, other *Staphylcoccus* spp, and others. For the sensitivity and specificity analyses, the samples with no growth and coliforms were combined to form a single category considered to be culture negative (negmod = 1). A single negmod = 1 entry would be formed from either a no-growth or a coliform isolation. Coliform isolates were considered to be contaminants, because the expected prevalence is usually < 2%. All other isolates were considered to be culture positive (negmod = 0).

The dairy was an open-shed, free-stall operation with sand bedding. Cows were milked twice daily in a double-18, rapid-exit parlor. Cow numbers on the dairy increased from < 1,200 cows to about 1,325 cows during the year. Each cow was individually identified. Nonlactating-cow antibiotic treatment had not been used for 3 years in this herd. The yearly cull rate was approximately 35%. Daily milk production ranged from 55 to 60 lb/cow. The bulk tank SCC ranged from about 180 $\times$ 10^3 to 300 $\times$ 10^3 cells/ml (Table 1). Individual cow composite SCC were obtained from the North Central Valley Dairy Herd Improvement Association. The SCC refers specifically to the SCC from the lactation immediately preceding calving. In the analyses, the last SCC for the lactation prior to beginning of the nonlactating period is indicated by Last. The highest SCC recorded during the lactation is reported as Max. The average SCC for the SCC re-

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Table 1—Monthly bulk tank somatic cell count (SCC) for a dairy herd during 1993

Month	SCC (cells × 10^3/ml)	Month	SCC (cells × 10^3/ml)
January	200	July	300
February	210	August	280
March	250	September	250
April	270	October	210
May	290	November	180
June	220	December	200

Table 2—Descriptive statistics of SCC (× 10^3/ml) for 515 cows in the study by general results of microbiologic analysis

	Negmod = 1			Negmod = 0		
Statistic	Last	Max	Avg	Last	Max	Avg
Mean	189	586	155	347	781	205
SE	20	52	10	50	73	15
Median	94	292	99	126	333	126
SD	304	779	148	874	1261	262
Range	3303	4751	849	9983	9951	2165
Min	13	32	21	16	48	27
Max	3316	4873	870	9999	9999	2192
Total	220	220	220	295	295	295

Negmod = 1, no-growth samples; Negmod = 0, growth sample; Last = last SCC prior lactation; Max = highest SCC prior lactation; Avg = mean SCC all prior lactation.

Table 3—Table used to calculate Sen, Spec, PV+, and PV−

SCC threshold	Culture positive (Negmod = 0)	Culture negative (Negmod = 1)
SCC above	True positive	False positive
SCC below	False negative	True negative

Sen = True positives/total culture positives; Spec = true negatives/total culture negatives; PV+ = true positives/total SCC above the threshold; and PV− = true negatives/total SCC below the threshold.

corded during the lactation is denoted by Avg. Unless otherwise noted, means are arithmetic means (Table 2).

Sensitivity is defined as the proportion of culture-positive cows that have SCC above the SCC threshold. Specificity is defined as the proportion of culture-negative cows that have SCC below the SCC threshold. A designated scheme was used for these determinations (Table 3). Predictive-value positive is the probability that a cow with a positive SCC test result is culture positive. Predictive-value negative is the probability that a cow with a negative SCC test result is culture negative.

Results

Only cows with a completed lactation, nonlactating period, and calving were included in the analysis. Of the cows in the herd, 515 cows met the inclusion criteria and had SCC from the preceding lactation and milk culture data at calving. Distribution of the cows by lactation was: lactation 1, 211 cows; lactation 2, 161 cows; and lactation ≥ 3, 140 cows. Lactation number was not available for 3 cows.

Results of microbiologic analysis yielded: 219 (42.8%), no-growth/coliforms; 108 (21.1%), other *Streptococcus* spp; 148 (28.9%), other *Staphylcoccus* spp; 10 (2.0%), *Streptococcus* spp and other *Staphylcoccus* spp; and 27 (5.3%), others. The others category included *Actinomyces, Proteus, Pasteurella,* and *Actinobacillus* spp and a single *Sta aureus*. No mycoplasmas or *Str agalactiae* were found.

For the samples with growth (negmod = 0), Last SCC was 347 × 10^3 ± 874 × 10^3 cells/ml (mean ± SD), compared with 189 × 10^3 ± 304 × 10^3 cells/ml for the no-growth category (negmod = 1). The same comparison for mean Max SCC was 586 × 10^3 ± 779 × 10^3 cells/ml for no-growth and 781 × 10^3 ± 1,261 × 10^3 cells/ml for growth categories. Mean Avg SCC for no-growth was 155 × 10^3 ± 148 × 10^3 cells/ml and 205 × 10^3 ± 262 × 10^3 cells/ml for growth categories (Table 2).

Discussion

It has been suggested that a sensitivity and specificity analysis is a valid evaluation for any test in any species. The threshold or critical value depends on the element of the test that is most important to optimize.[7] This analysis is particularly relevant when using a surrogate test instead of a pathognomonic test.[5] In our study, the SCC was a surrogate test for mastitis. The appropriate pathognomonic test or standard is microbiologic testing or culture and identification of causative organisms in milk samples taken from the cows. For our purposes, we wanted to verify whether, at a given threshold SCC, various SCC combinations could be used to detect a culture-positive sample (ie, mastitis) from a cow. To validate the SCC test, we determined the percentage of infected and noninfected cows that were correctly identified by SCC at a given threshold value. Sensitivity pertains to the infected cows, and specificity relates to the noninfected cows. As always, the relation between sensitivity and specificity changes in an inverse fashion; the threshold is varied because of overlapping SCC distribution of the infected and noninfected cows.

The monthly bulk tank SCC was determined for the dairy herd during 1993 (Table 1). The monthly bulk tank SCC was 238 × 10^3 ± 40 × 10^3 cells/ml (mean ± SD) with a median of 235 × 10^3 and a range of 120 × 10^3 (180 to 300 × 10^3 cells/ml). Thus, this dairy was classified as having a herd with low SCC. This is an important factor because the predictive value of a test will vary with prevalence of disease animals.[5,7,8] In this instance, prevalence is low. In other instances, where prevalence is high, the predictive value would probably be higher than that in our study herd. For this reason generalized recommendations for threshold SCC and the predictive value should not be made.[7]

We combined the no-growth and coliform groups into a single category (negmod = 1) for the data analyses, because milk samples were collected only from cows without signs of clinical disease (ie, normal milk, no sudden decrease in milk production, and not identified for isolation in hospital group). Because the expected percentage of coliform-infected cows without clinical signs of disease is low (1 to 2% point prevalence),[9] even with the maximal expected coliform prevalence, our calculations would only be slightly affected.

Results of a previous study[10] indicated that the predictive value of SCC varies with the organisms causing

Table 4—Frequency distribution of SCC by Last, Max, and Avg values, and culture results for the total herd

SCC	Negmod = 1			Negmod = 0		
	Last*	Max	Avg	Last	Max	Avg
0–100	113(52)	27(12)	112(51)	124(24)	24(8)	121(41)
101–200	51(23)	47(21)	45(26)	72(25)	63(22)	92(31)
201–300	24(11)	42(19)	18(8)	34(12)	42(14)	30(10)
301–500	15(7)	35(16)	23(11)	24(8)	57(19)	27(9)
≥ 501	16(7)	68(31)	10(5)	39(13)	107(37)	23(8)
Total	219	219	219	293	293	293

*Number (percentage of column total).
See Table 2 for key.

Table 5—Results of total herd analyses for the Last, Max, and Avg SCC values

Threshold	Sen	Spec	PV+	PV–
Last SCC (× 10^3 cells/ml)				
100	0.58	0.52	0.61	0.48
200	0.33	0.75	0.64	0.46
300	0.22	0.86	0.67	0.45
500	0.13	0.93	0.71	0.44
Max SCC (× 10^3 cells/ml)				
100	0.92	0.12	0.58	0.53
200	0.70	0.34	0.59	0.46
300	0.56	0.53	0.62	0.47
500	0.37	0.69	0.61	0.45
Avg SCC (× 10^3 cells/ml)				
100	0.59	0.51	0.62	0.48
200	0.27	0.77	0.61	0.44
300	0.17	0.85	0.60	0.43
500	0.08	0.95	0.70	0.43

See Tables 2 and 3 for key.

Table 6—Results of first-lactation analyses for the Last, Max, and Avg SCC values

Threshold	Sen	Spec	PV+	PV–
Last SCC (× 10^3 cells/ml)				
100	0.41	0.71	0.61	0.51
200	0.21	0.86	0.63	0.49
300	0.09	0.93	0.59	0.47
500	0.04	0.96	0.71	0.47
Max SCC (× 10^3 cells/ml)				
100	0.87	0.20	0.55	0.57
200	0.56	0.43	0.53	0.47
300	0.36	0.61	0.51	0.45
500	0.20	0.76	0.46	0.48
Avg SCC (× 10^3 cells/ml)				
100	0.37	0.65	0.54	0.47
200	0.12	0.88	0.54	0.47
300	0.08	0.91	0.50	0.47
500	0.03	0.96	0.43	0.47

See Tables 2 and 3 for key.

Table 7—Results of second-lactation analyses for Last, Max, and Avg SCC

Threshold	Sen	Spec	PV+	PV–
Last SCC (× 10^3 cells/ml)				
100	0.66	0.40	0.61	0.46
200	0.35	0.70	0.62	0.44
300	0.24	0.79	0.62	0.42
500	0.17	0.91	0.72	0.43
Max SCC (× 10^3 cells/ml)				
100	0.93	0.09	0.59	0.46
200	0.74	0.31	0.60	0.46
300	0.61	0.54	0.65	0.49
500	0.41	0.69	0.65	0.45
Avg SCC (× 10^3 cells/ml)				
100	0.67	0.48	0.64	0.51
200	0.32	0.72	0.61	0.43
300	0.19	0.87	0.67	0.43
500	0.07	0.99	0.88	0.43

See Tables 2 and 3 for key.

Table 8—Results of third-lactation and greater analyses for Last, Max, and Avg SCC

Threshold	Sen	Spec	PV+	PV–
Last SCC (× 10^3 cells/ml)				
100	0.70	0.30	0.62	0.38
200	0.46	0.60	0.66	0.41
300	0.34	0.81	0.75	0.43
500	0.21	0.89	0.75	0.41
Max SCC (× 10^3 cells/ml)				
100	0.98	0.02	0.62	0.33
200	0.85	0.19	0.63	0.43
300	0.66	0.38	0.63	0.50
500	0.53	0.57	0.70	0.42
Avg SCC (× 10^3 cells/ml)				
100	0.78	0.30	0.62	0.46
200	0.41	0.62	0.64	0.39
300	0.26	0.72	0.61	0.37
500	0.15	0.91	0.72	0.39

See Tables 2 and 3 for key.

the infections. The predictive value of increased SCC is better for the contagious or major pathogens, compared with environmental pathogens. In our study herd where environmental pathogens were almost exclusively involved, we anticipated a poor ability of the SCC to predict infection, as had been suggested.[5] The environmental source of most infections within this study herd also was another consideration for poor predictability owing to the characteristics of the infections.

Our study herd had high prevalence of environmental pathogens on the basis of their culture and identification at calving; prevalence was 56.9% (292/515) for all cows that calved. The environmental *Streptococcus* spp prevalence was 22.9% (108 + 10 with *Streptococcus* and coagulase-negative *Staphylcoccus* spp) and was 30.7% (148 + 10 with *Streptococcus* and coagulase-negative *Staphyloccus* spp). In a Canadian study,[11] the prevalence at calving for coagulase-negative *Staphylcoccus* spp was reported to be 4.8% for pure-culture and 13.8% for mixed-culture results. Those authors suggested that coagulase-negative *Staphylcoccus* spp are the most common organisms isolated from the milk of dairy cows. A Kentucky study[12] determined prevalence of 35.5% for coagulase-negative *Staphylcoccus* spp. They indicated that coagulase-negative *Staphylcoccus* spp may be the most prevalent organisms isolated from herds, using the currently recommended mastitis control measures.

It is not surprising that the prevalences vary so widely among these studies, because environment and management are different in these 3 areas. Moreover, use of differing postmilking teat dipping has been documented to cause variation in the isolation of these organisms.[3]

Frequency distribution was determined for the SCC according to Last, Max, and Avg SCC (Table 4) for the total herd on the basis of culture results as negmod = 0 or negmod = 1. The sensitivity, specificity, predictive-value positive, and predictive-value neg-

ative for the total herd and by lactation groups (1, 2, and ≥ 3) also were determined (Tables 5–8).

The Avg SCC for infected cows in this herd was $205 \times 10^3 \pm 262 \times 10^3$ cells/ml (mean ± SD). In a previous Canadian study,[11] a comparable SCC of 251×10^3 cells/ml was reported. In a Wisconsin study,[13] the mean average composite SCC was 232×10^3 cells/ml. In most reports, the SCC for the environmental pathogens, such as those isolated by us, have been higher than those isolated from noninfected cows but lower than those isolated from infected cows, from which major, contagious pathogens were isolated.[3,11,13] Mean Avg SCC for our negmod = 1 cow was $155 \times 10^3 \pm 148 \times 10^3$ cells/ml. In other studies,[11,13] the noninfected cows were found to have composite SCC of 186×10^3 cells/ml. Our results seem comparable to those of previous reports.

None of the various SCC (Last, Max, or Avg) thresholds will provide adequate sensitivity and specificity on which to predict the infectious status of cows at calving in this herd. For example, a dairy producer might want to selectively apply nonlactating-cow antibiotic treatment on the basis of SCC. If a threshold SCC value $\geq 100 \times 10^3$ cells/ml for the last SCC test was selected, the sensitivity would be 0.58 and the specificity would be 0.52. Using these criteria, only 57% of the infected cows would be correctly identified (true positives) for treatment. The remaining 43% (false positives) would not be identified for treatment when they should be treated. Of the cows that were not infected, 48% (false negatives) would be identified as infected and selected for treatment when it was not needed. The remaining cows (true negatives) would be correctly identified at this threshold and selected for treatment. Many cows that should have been treated would remain possibly infected into the next lactation, and the expense of treating cows with no infections would be wasted. Under those circumstances, selective treatment does not seem feasible.

Use of SCC to predict the infection status of cows at calving would be of considerable interest so that producers could use selective nonlactating-cow antibiotic treatment instead of treating all quarters of cows at the beginning of the nonlactating period. Such antibiotic treatment has been one of the principal recommendations for controlling contagious mastitis for decades.[3,14] Its aim is to reduce prevalence of infections carrying over from one lactation to the next. However, results of a recent Ontario, Canada, study[1] indicated that 28% of a random sample of dairymen using their Dairy Herd Improvement SCC option (average bulk tank SCC, 245×10^3 cells/ml) were selectively using nonlactating-cow treatment. About 40% were using this treatment on the basis of high SCC. These producers had herds with significantly lower SCC; however, a cause-effect relation was not determined. Our attempt was to find a method for predicting cows that should be treated at the beginning of the nonlactating period.

Our study at this California dairy with low SCC and high prevalence of gram-positive, environmental mastitis pathogens indicated that it was not possible to predict the infection status of cows at calving by using SCC from the preceding lactation. At least for the herd studied, a program of selective nonlactating-cow treatment on the basis of any combination of SCC cannot be rationally recommended.

References

1. Ravinderpal G, Howard WH. Economics of mastitis control. *J Dairy Sci* 1990;73:3340–3348.
2. Nonlactating cow therapy. *National Mastitis Council bulletin C12254*. Arlington, Va: National Mastitis Council, 1993.
3. Harmon RJ, Langlois BE. Mastitis due to coagulase-negative Staphylococcus species. *Agri-Pract* 1989;10:29–34.
4. Nickerson SC. Mastitis and its control in heifers and nonlactating cows. In: *International symposium on bovine mastitis*. Arlington, Va: National Mastitis Council, 1990;82–91.
5. Martin SW, Meek AH, Willeberg P. Detecting subclinical disease with screening tests. In: *Veterinary epidemiology: principles and methods*. Ames, Iowa: Iowa State University Press, 1987;62–66.
6. Dohoo IR. Evaluating mastitis tests, in *Proceedings*. Annu Meet Natl Mastitis Counc 1990;68–75.
7. McDermott MP, Erb HN, Natzke RP. Predictability by SCC related to prevalence of intramammary infection within herds. *J Dairy Sci* 1982;65:1535–1539.
8. Thurmond MC. Epidemiologic methods in mastitis treatment and control. *Vet Clin North Am Food Anim Pract* 1993;9:435–444.
9. Smith KL, Hogan JS. Environmental mastitis. *Vet Clin North Am Food Anim Pract* 1993;9:489–498.
10. Sears PM, Gonzales RN, Wilson DJ, et al. Procedures for mastitis diagnosis and control. *Vet Clin North Am Food Anim Pract* 1993;9:445–468.
11. Davidson TJ, Dohoo IR, Donald AW, et al. A cohort study of coagulase negative Staphylococcal mastitis in selected dairy herds in Prince Edward Island. *Can J Vet Res* 1992;56:275–280.
12. Matthews KR, Harmon RJ, Langlois BE. Prevalence of *Staphylococcus* species during the periparturient period in primiparous and multiparous cows. *J Dairy Sci* 1992;75:1835–1839.
13. Timms LL, Sommer DA, Schultz LH. Role of minor pathogens in mastitis. *J Dairy Sci* 1985;68[Suppl 1]202.
14. Kirk JH, DeGraves F, Tyler J. Recent progress in treatment and control of mastitis in cattle. *J Am Vet Med Assoc* 1994;204:1152–1158.