Utility of an in-line somatic cell count sensor for selecting cows for dry-cow therapy

S. McDougall[1] and R. Orchard[2]

[1] Cognosco, Anexa Veterinary Services, Morrinsville, New Zealand
[2]MSD Animal Health, Upper Hutt, New Zealand

Abstract

Minimizing the use of antimicrobials at the end of lactation (dry cow therapy, DCT) requires categorization of cows as likely infected or uninfected. While microbiology is the gold standard, indirect tests such as somatic cell count (SCC) are commonly used. An in-line SCC sensor (SenseHub In Line Somatic Cell Count, in-line SCC) is commercially available but its utility to differentiate cows eligible for dry cow therapy has not been assessed. This study tested the utility of in-line SCC to select cows for different dry-off treatments, using cow-composite milk samples submitted for conventional microbiology as the gold standard.

A secondary objective was to compare the utility of in-line SCC with the maximum (max DHI SCC) or last (last DHI SCC) SCC results from monthly DHI tests. Cows (n = 1,544) from four New Zealand herds had cow-composite milk samples collected using aseptic methods at the final milking of lactation and submitted for microbiology testing. Microbiology data from approximately half the cows (n = 770; training dataset) were used to determine the optimal predictor for indicating intramammary infection (IMI) from the in-line SCC data, which was the twelve-week average SCC (in-line 12wSCC). Using the data from the remaining cows (test dataset), the area under receiver-operator curve (AUC) for max and last DHI SCC were compared with that of the in-line 12wSCC. The AUC for a major pathogen IMI was 0.82, 0.82 and 0.84 for in-line 12wSCC, max DHI SCC and last DHI SCC, respectively. These AUC did not differ and the AUC for the in-line 12wSCC was non-inferior to that of the last and maximum HT SCC (both $P < 0.001$). It was concluded that the in-line 12wSCC had AUC, sensitivity and specificity not different from DHI SCC data and hence this test has utility in selecting cows for different dry cow therapy treatments.

Introduction

Due to concerns about antimicrobial use and the risk of selection for resistant pathogens, dairy farmers around the world are under pressure to reduce antimicrobial usage. Treatment of clinical mastitis cases during lactation and infusion of antimicrobials at the end of lactation (dry cow therapy, DCT) constitutes the majority of antimicrobial usage in dairy cows (Schrag *et al.,* 2020). However, not all cows and quarters have an intramammary infection (IMI) at the end of lactation, with the quarter-level prevalence of IMI reported as ranging between 13 and 61% (Pantoja *et al.,* 2009, Bradley *et al.,* 2011, Gohary and McDougall, 2018). Hence categorizing cows as unlikely to be infected and hence not requiring antimicrobial DCT (selective DCT) enables antimicrobial usage to be optimized to manage herd mastitis.

Cow-composite SCC using a threshold of >200,000 cells/mL has a sensitivity (Se) and specificity (Sp) of 83.4 % and 58.9%, respectively, for identification of a major

pathogen IMI, and 72.6%, and 85.5%, for identification of any IMI (Timms and Schultz, 1987, Dohoo and Leslie, 1991). Not all producers regularly receive individual cow SCC as part of production recording. Other methods commonly used as a basis for selective DCT are milk culturing (Cameron *et al.,* 2014, Kabera *et al.,* 2020, Rowe *et al.,* 2020) and the California Mastitis Test (CMT) (Timms and Schultz, 1987, Pyörälä, 2003), however these methods are labour intensive, require training and, in the case of culturing, expensive.

An automatic in-line detection system (SenseHub In Line Somatic Cell Count) based on assessing the viscosity of milk following addition of a detergent has been developed (Whyte *et al.,* 2004). The in-line detection system provides longitudinal monitoring of cows that can potentially differentiate cows eligible for DCT.

The objective of the current study was to assess the association between the SCC estimated by the in-line sensor and the presence of IMI at the end of lactation. A secondary objective was to assess the relative utility of DHI cow-composite SCC and the in-line SCC estimates in differentiating cows eligible for DCT.

Materials and methods

Herds and cows

Data from 1,544 cows from four New Zealand spring calving, predominantly pasture-fed dairy herds were analyzed. Herds varied from 281 to 1,023 cows and were predominantly Friesian-Jersey crossbreds. All herds had in-line SCC sensors installed (SenseHub In-Line Somatic Cell Count, MSD Animal Health, Hamilton, New Zealand), and undertook monthly production (DHI) recording including cow-composite SCC (i.e. DHI SCC, LIC New Zealand). Herds were milked twice daily for most of the lactation, but all herds were milked once-a-day for some weeks prior to drying off.

In-line SCC sensors

The in-line sensor used in this study has previously been validated and shown to provide estimates of ten-day average individual cow SCC at least as precisely as a single-day DHI test in the middle of the ten-day period (Orchard *et al.,* 2018).

The in-line SCC sensors were mounted in a subset of the long milk tubes in the parlor such that between 26 and 50% of milking-points had the units in place. The sensors automatically draw off a sample of milk 30 to 60 seconds after the start of milking for analysis and report an estimated somatic cell count. One of the four farms (Herd 1) chose to turn the units on only in late lactation to generate data for the current study, for the last 28, 44 or 45 days of lactation, depending on the cows' dry-off dates.

Sampling and microbiology

On the last day cows were milked for the lactation, trained technicians aseptically collected approximately 1 mL of milk from each quarter into the same vial. Samples were frozen from the day of collection until culturing (within 14 days of the last day of sampling for a herd). Microbiology was undertaken in the diagnostic laboratory of Cognosco (Morrinsville, New Zealand) following modified National Mastitis Council procedures (Middleton *et al.,* 2017).

Statistical analysis

Cows were categorized as being IMI positive (i.e. presence of any bacterial species including both minor and major pathogens) or negative, and as having a major pathogen IMI or not. *Staphylococcus aureus*, *Streptococcus dysgalactiae* or *Streptococcus uberis* were defined as major pathogens.

The statistical analysis was based on the two study objectives (that is, the association between the in-line 12wSCC and IMI, and the correlation between classification of IMI based on the in-line 12wSCC and classification based on DHI SCC), using the cow-composite IMI data as the reference (i.e. the gold standard) test.

Within each farm, each cow was categorized as IMI-positive or IMI-negative and within each category, cows were ranked on the most recent (last) DHI SCC result and randomly assigned in sequential pairs to either a test or training dataset. The training dataset was used to optimize the in-line SCC decision criteria, and the test dataset was used for estimating its test characteristics and for comparison with DHI SCC.

The bacteriological results for the training dataset were disclosed to one of the authors (RO) and this dataset was used to assess the test characteristics of several potential predictors derived from the in-line SCC data. Predictors created and examined included: maximum, geometric mean, and bounded geometric mean of in-line SCC results in the last one, two, four, six, eight, and 12 weeks of lactation. The bounded geometric mean was created by substituting a value of 50,000 cells/mL for values of <50,000 cells/mL prior to calculating the geometric mean. A series of receiver operator curves (ROC) were undertaken assessing each of the above defined variables against bacteriology at dry off as the gold standard. The variable with the maximum area under the curve (AUC) was then used as the in-line predictor for further analyses.

The cut-point at which the sum of the sensitivity and specificity were maximized (i.e. the Youden index) was calculated. The association between the in-line 12wSCC and presence of any or a major IMI was assessed by creating 2 x 2 tables and computing the 95% confidence intervals for Se, Sp, negative predictive value (**NPV**), positive predictive value (**PPV**), and accuracy (i.e. the sum of true positive and true negative divided by total number of tests).

The SCC and date of the last DHI SCC of lactation and the maximum DHI SCC across lactation were selected for each cow. If the DHI SCC at the last herd test of lactation was missing, the preceding DHI SCC result for the cow was used.

The association between the last and maximum DHI SCC and presence of any, or a major IMI were assessed by creating 2 x 2 tables and computing the exact 95% confidence intervals for Se, Sp, NPV, PPV and accuracy.

The AUC of the ROC of the 3 tests (in-line 12wSCC, maximum DHI SCC, last DHI SCC) were directly compared using the “rocomp” command in STATA. Non-inferiority testing of the AUC of the ROC for the test dataset of cows of the in-line 12wSCC relative to either the maximum and last DHI SCC was undertaken for any IMI and for major pathogen IMI using the non-inferiority algorithm implemented in the rocNIT package within R (Liu *et al.,* 2006), with a delta of 0.1.

The analysis in this section was undertaken in STATA version 17.0 (STAT Corp, College Station, Texas, USA) other than the AUC non-inferiority analyses which were undertaken within R Studio version 1.3.1093 (2020).

Results

The cow-level prevalence of minor and major IMI was 50.6% and 14.2%, respectively and the proportion of cows within herds infected differed (P < 0.001). The prevalence of Corynebacterium spp., CNS, Staph aureus, Strep dysgalactiae, Strep uberis, mixed major IMI (i.e., one or more major pathogens and at least one other bacterial species), mixed minor IMI (i.e. a Corynebacterium spp. and CNS) or another spp. were 16.3%, 26.9%, 3.9%, 0.1%, 4.8%, 5.5%, 6.8% and 0.6%, respectively.

From the training dataset, the AUC was maximal (0.81 (95% CI 0.77-0.85)) when the bound geometric mean of the last 12 weeks of in-line SCC data ("in-line 12wSCC") was used. Hence, this summary variable was used for the subsequent analyses.

The median (25th-75th percentile) of the number of in-line SCC data points over the last 12 weeks of lactation was 18 (13-22). The in-line 12wSCC was 630 (SD 461) for major pathogens, 318 (SD 310) for minor pathogens, and 143 (SD 109) for no growth x 1,000 cells/mL (P < 0.001; Figure 1). The in-line 12wSCC Se and Sp were 0.68 and 0.71, respectively for any IMI, and 0.89 and 0.51, respectively, for a major IMI, when a cut-point of 150,000 cells/mL was used (Table 1).

Using the same cut-point, the Se and Sp for any IMI at the last DHI SCC was 0.68 and 0.68, respectively, and the Se and Sp for a major IMI for the last DHI SCC was 0.91 and 0.50, respectively (Table 2). The Se and Sp for any IMI for the maximum DHI SCC test was 0.82 and 0.52, respectively, and the Se and Sp for a major IMI at maximum DHI SCC was 0.99 and 0.35, respectively.

There was no difference in the AUC for predicting the presence of a major IMI using the natural log of the in-line 12wSCC (0.824), last DHI SCC (0.816) or the maximum lactational DHI SCC (0.836) (P = 0.50; Figure 2a; Table 3). The in-line 12wSCC AUC was noninferior to both the maximum and last DHI SCC for major IMI (both P < 0.001). The AUC for predicting presence of any IMI at drying off was greater using the in-line 12wSCC (0.776) than either the last DHI SCC (0.737) or the maximum DHI SCC (0.735) (both P = 0.03; Figure 2b; Table 3). The in-line 12wSCC AUC was noninferior to both the maximum and last DHI SCC for any IMI (both P < 0.001).

The cutpoint to achieve at least a 0.9 Se was ≥143,000 cells/mL for both the in-line 12wSCC and the last DHI SCC, and ≥269,000 cells/mL for the maximum DHI SCC. The cutpoint to achieve a maximum of the sum of the sensitivity and specificity (i.e.

Table 1. The sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), and accuracy, the standard error (SE) and 95% confidence intervals for cows with intramammary infection associated with any pathogen (upper panel) or having a major (Major; lower panel) pathogen intramammary infection categorized by the in-line 12wSCC using a cut-point of ≥ 150,000 cells/mL in the test population.

				95% CI	
		Mean	SE	Low	High
Any pathogen	Se	0.679	0.021	0.636	0.720
	Sp	0.710	0.028	0.652	0.763
	PPV	0.812	0.019	0.771	0.848
	NPV	0.545	0.026	0.492	0.598
	Accuracy	0.690	0.017	0.656	0.722
Major	Se	0.894	0.030	0.819	0.946
	Sp	0.512	0.019	0.473	0.550
	PPV	0.221	0.020	0.183	0.264
	NPV	0.969	0.009	0.945	0.984
	Accuracy	0.563	0.018	0.528	0.599

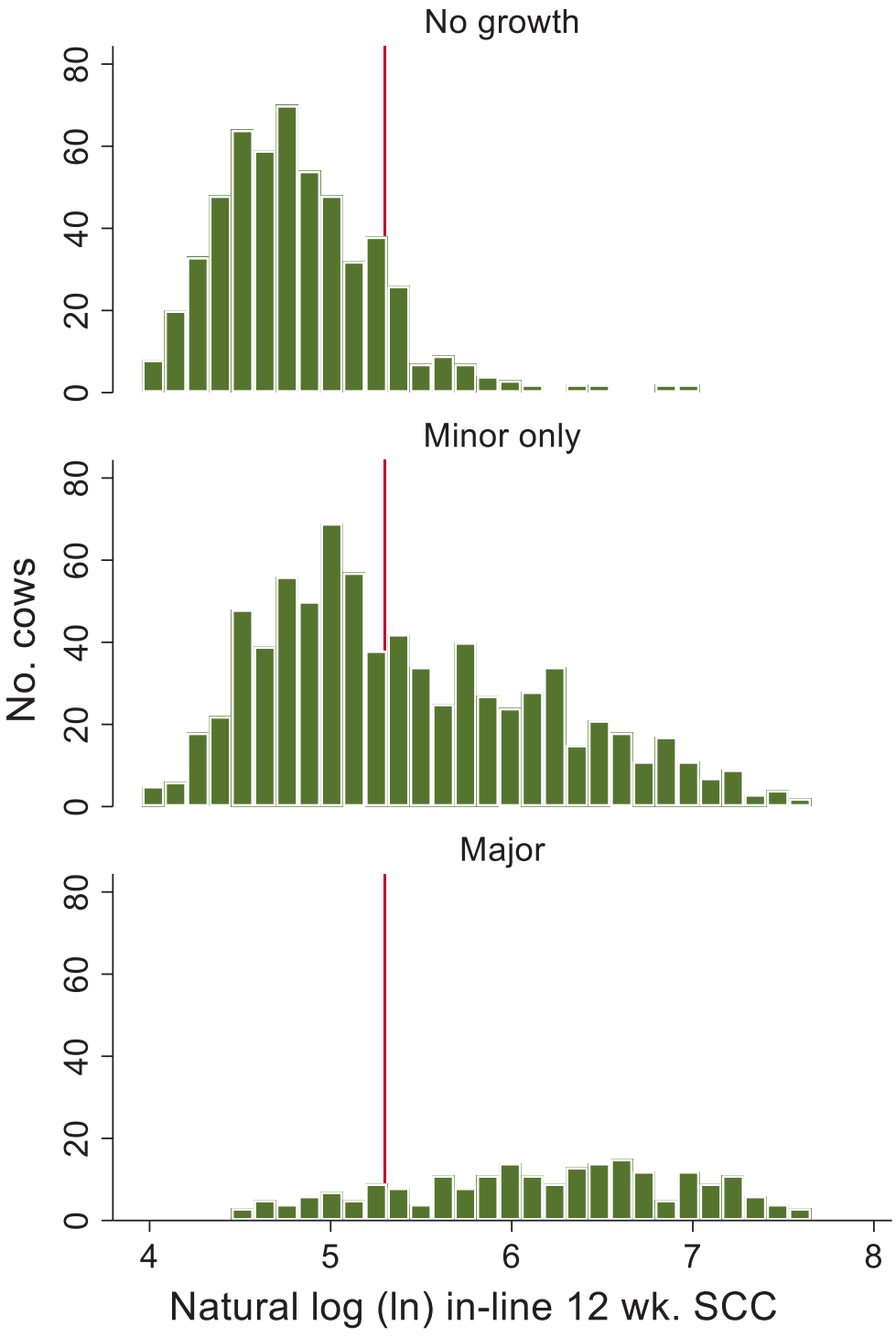

Figure 1: Frequency histogram of the natural log of the bounded 12-week geometric mean in-line SCC (x 1,000 cells/mL; in-line 12wSCC) by IMI bacterial pathogen at cow level. The vertical reference line is at 200,000 cells/mL.

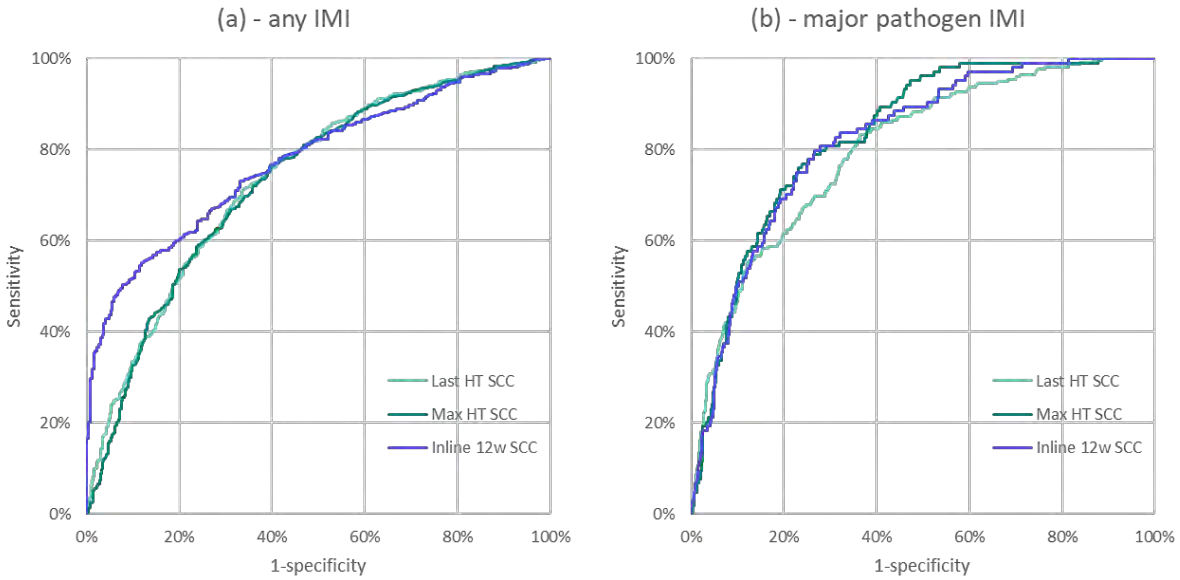

Figure 2: Receiver operator curve for presence of (a) a major pathogen or (b) any intramammary infection at drying off using either the bounded geometric mean of the in-line SCC over the last 12 weeks of lactation ("in-line 12wSCC"), the SCC at the last DHI herd test or the maximum DHI SCC across lactation. Only those cows in the test population were used for this analysis.

Table 2. The mean, standard error of the mean (SE) and 95% binomial confidence intervals for the sensitivity (Se), specificity (Sp) positive predictive value (PPV), negative predictive value (NPV), and accuracy using a cut-point of ≥ 150,000 cells/mL for the last herd test DHI SCC and the maximum herd test DHI SCC, using the cow-composite milk microbiology results as the gold standard.

					95% CI	
			Mean	SE	Low	High
Last DHI SCC	Any IMI	Se	0.683	0.021	0.641	0.724
		Sp	0.684	0.028	0.625	0.739
		PPV	0.800	0.019	0.758	0.836
		NPV	0.539	0.027	0.485	0.593
		Accuracy	0.683	0.017	0.649	0.716
	Major IMI	Se	0.913	0.028	0.842	0.960
		Sp	0.501	0.019	0.463	0.540
		PPV	0.221	0.020	0.183	0.264
		NPV	0.974	0.009	0.951	0.988
		Accuracy	0.557	0.018	0.521	0.592
Max DHI SCC	Any IMI	Se	0.817	0.017	0.780	0.850
		Sp	0.515	0.030	0.454	0.575
		PPV	0.756	0.018	0.718	0.792
		NPV	0.603	0.032	0.537	0.667
		Accuracy	0.711	0.016	0.677	0.742
	Major IMI	Se	0.990	0.010	0.948	1.000
		Sp	0.345	0.018	0.309	0.382
		PPV	0.190	0.017	0.158	0.226
		NPV	0.996	0.004	0.976	1.000
		Accuracy	0.432	0.018	0.396	0.467

Table 3. The estimated area under the curve (AUC), the standard error of the estimate of the area under the curve (SE) and the 95% confidence interval for the area under the curve where the bounded geometric mean of in-line SCC during the last 12 weeks of lactation (in-line 12wSCC), the somatic cell count at the last herd test of lactation (Last HT SCCLast DHI SCC) or the maximum lactational herd test SCCDHI SCC (Max HT SCCMax DHI SCC) were used to predict the presence of a major and any intramammary infection at drying off for cows in the test population (n = 773 cows).

				95% CI	
		AUC	SE	Low	High
Major IMI	In-line 12wSCC	0.824	0.020	0.785	0.863
	Last DHI SCC	0.816	0.021	0.776	0.857
	Max DHI SCC	0.836	0.019	0.800	0.873
Any IMI	In-line 12wSCC	0.776	0.016	0.744	0.808
	Last DHI SCC	0.737	0.019	0.700	0.774
	Max DHI SCC	0.735	0.019	0.698	0.772

the Youden index) was higher than that required to achieve a 0.9 Se for all three SCC measurements.

Discussion

This study assessed the test characteristics of an in-line SCC sensor and compared the test performance with use of the last or maximum DHI SCC in determining the presence of any, or of a major IMI at the end of lactation. The bounded geometric mean of the in-line SCC recorded over the last 12 weeks of lactation (in-line 12wSCC) was found to be the best predictor of intramammary infection. Based on the area under the curve following receiver operator curve analyses, the in-line 12wSCC predictions were found to be not different from, and not inferior to, either the last or the maximum DHI SCC.

The four herds enrolled in the current study were distributed across the key dairying regions of New Zealand and the range of herd size, clinical mastitis incidence and bulk tank SCC were broadly in line with the New Zealand industry. Additionally, the prevalence of IMI and pathogens present at drying off were similar to that reported by previous New Zealand studies (McDougall, 2010, McDougall *et al.,* 2021). The estimates of the AUC were similar, the Se was higher, and Sp lower in the current study for last or maximum DHI SCC as a predictor compared to a previous New Zealand study which enrolled cows from 36 herds across four regions of New Zealand (McDougall *et al.,* 2021). Herds in that previous study had only four DHI tests, suggesting that availability of an increasing number of SCC data points may improve sensitivity of detection of IMI. Overall, the external validity of the study for the New Zealand context appears strong.

The median number of data points over the last 12 weeks of milking for the in-line 12wSCC was 18. This was lower than the expected value of 42 which assumes that cows were milked twice a day and that there was 25% bail coverage, in which case a data point would be generated every 2nd day on average. The number is lower than expected due to herds milking cows only once a day prior to drying off and having the sensors turned off to save consumable costs in one herd. Despite the lower-than-expected number of data points, the in-line 12wSCC was moderately informative for the presence of any IMI, and highly informative for the presence of a major IMI as the AUC were approximately 0.71 and 0.92, respectively. An AUC of between 0.7 and

0.9 is moderately informative, while an AUC > 0.9 is regarded as highly informative (Swets, 1988). The Se and Sp and hence AUC were lower when the outcome was any IMI compared with where only presence of a major IMI was considered. This is associated with the high prevalence of minor pathogens and the lower SCC levels in minor IMI-cows.

The test characteristics of the in-line 12wSCC were similar to those using the last or maximum DHI SCC data. While using mean data over 12 weeks may have resulted in short-term increases in SCC being obscured, this does not appear to have negatively impacted the test characteristics of the in-line 12wSCC. Short-term increases in SCC associated with IMI which subsequently self-cure may be missed by averaging. However, if the animal self-cures, then our gold standard test of presence of IMI at drying off would classify these animals as uninfected, as would the lower geometric mean SCC from the in-line sensor. Evaluating shorter periods of in-line SCC data did not improve the AUC for detection of IMI. The trade-off between potentially increased sensitivity for short term increases in SCC when using shorter periods may be offset by reduced accuracy associated with having fewer SCC data points upon which to make decisions.

Herds in this study had monthly herd tests, resulting in between 8 and 9 herd tests for each herd. Thus, the number of herd test data points for each cow in the current study was greater than the New Zealand practice of undertaking 3 or 4 DHI tests per lactation. This increased number of DHI tests may have resulted in higher Se in detecting short-term elevations in SCC associated with infection in the current study compared to industry standard. However, the AUC using maximum DHI SCC in the current study for detection of major IMI was 0.84 (SE 0.02), which is an identical estimate to that from a previously published New Zealand study using two-monthly herd test data (McDougall *et al.,* 2021). This suggests that increasing frequency of DHI SCC from two-monthly to monthly does not materially increase the ability of maximum DHI SCC to define IMI at the end of lactation.

It is concluded that the bounded geometric mean of in-line SCC data for the last 12 weeks of lactation is equivalent and noninferior to the use of DHI SCC from the entire lactation (i.e. the maximum SCC) or the SCC from the last herd test. Hence, In-line SCC has utility for selecting cows for either antimicrobial or internal teat sealant treatment at the end of lactation.

Acknowledgements

Derived from Journal of Dairy Science, 107/12, Scott McDougall, Amanda Kilby, Jo Holter, Rob Orchard, Utility of an in-line somatic cell count sensor for selecting cows for dry-cow therapy, 11342-11352, Copyright (2024), with permission from Elsevier.

Jo Holter and Amanda Kilby of MSD Animal Health made invaluable contributions to the organisation and design of this study. The herd owners and their staff are thanked for their support. The support of Cognosco and Anexa technicians including Elizabeth Blythe, Courtney Boersen, Cathy Yanez, and Bev Brownlie in collecting milk samples and data recording is gratefully acknowledged. Tiarna Graham, Laura Dalzell, Caitlin Bell, Mandi Beatie, and Anna Lauts undertook sampling and data recording on behalf of VetSouth. JP LeProu, Nathan Potter, Ondine Habgood, Paddy Stevenson, and Peggy Lawn undertook the recording and sampling at the Taranaki (Coastal Vets) site. The bacteriology work of Yvette MacPherson and Ali Karkaba of Cognosco, Anexa Veterinary Services is gratefully acknowledged.

List of references

Bradley, A. J., J. E. Breen, B. Payne, and M. J. Green. 2011. A comparison of broad-spectrum and narrow-spectrum dry cow therapy used alone and in combination with a teat sealant. J. Dairy Sci. 92:692-704. (https://doi.org/10.3168/jds.2010-3192).

Cameron, M., S. Mckenna, K. Macdonald, I. Dohoo, J. Roy, and G. Keefe. 2014. Evaluation of selective dry cow treatment following on-farm culture: Risk of postcalving intramammary infection and clinical mastitis in the subsequent lactation. J. Dairy Sci. 97:270-284. (https://doi.org/10.3168/jds.2013-7060).

Dohoo, I. R. and K. E. Leslie. 1991. Evaluation of changes in somatic cell counts as indicators of new intramammary infections. Prev. Vet. Med. 10:225-237. (https://doi.org/10.1016/0167-5877(91)90006-N).

Gohary, K. and S. McDougall. 2018. Predicting intramammary infection status at drying off using indirect testing of milk samples. N. Z. Vet. J. 66:312-218. (https://doi.org/10.1080/00480169.2018.1509741).

Kabera, F., S. Dufour, G. Keefe, M. Cameron, and J.-P. Roy. 2020. Evaluation of quarter-based selective dry cow therapy using Petrifilm on-farm milk culture: A randomized controlled trial. J. Dairy Sci. 103:7276-7287. (https://doi.org/10.3168/jds.2019-17438).

Liu, J.-P., M.-C. Ma, C.-y. Wu, and J.-Y. Tai. 2006. Tests of equivalence and non-inferiority for diagnostic accuracy based on the paired areas under ROC curves. Stat. Med. 25:1219-1238. (https://doi.org/10.1002/sim.2358)

McDougall, S. 2010. A randomised, non-inferiority trial of a new cephalonium dry cow therapy. N. Z. Vet. J. 58:45-58. (https://doi.org/10.1080/00480169.2010.65060).

McDougall, S., J. Williamson, K. Gohary, and J. Lacy-Hulbert. 2021. Detecting intramammary infection at the end of lactation in dairy cows. J. Dairy Sci. 104:10232-10249. (https://doi.org/10.3168/jds.2020-20036).

Middleton, J. R., L. K. Fox, G. Pighetti, and C. Petersson-Wolfe. 2017. Laboratory Handbook on Bovine Mastitis 3rd ed. NMC, New Prague, MN, USA.

Orchard, R. G., G. Anderson, and A. Winkelman. 2018. Comparison of individual cow SCC estimates using an on-line SCC analyser and conventional herd tests. ICAR Technical Series 23:37-43.

Pantoja, J. C. F., C. Hulland, and P. Ruegg. 2009. Dynamics of somatic cell counts and intramammary infections across the dry period. Prev. Vet. Med. 90:43-54. (https://doi.org/10.1016/j.prevetmed.2009.03.012).

Pepe, M. S., G. Longton, and H. Janes. 2009. Estimation and comparison of receiver operating characteristic curves. Stata J. 9:1-16. (https://doi.org/10.1177/1536867X0900900101).

Pyörälä, S. 2003. Indicators of inflammation in the diagnosis of mastitis. Vet. Res. 34:565-578. (https://doi.org/10.1051/vetres:2003026).

Rowe, S.M., S.M. Godden, D. V. Nydam, P. J. Gorden, A. Lago, A.K.-Vasquez, E. Royster, J. Timmerman, and M.J. Thomas. 2020. Randomized controlled trial investigating the effect of 2 selective dry-cow therapy protocols on udder health and performance in the subsequent lactation. J. Dairy Sci. 103:6493-6503. (https://doi.org/10.3168/jds.2019-17961).