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Investigation of differential somatic cell count as a potential new supplementary indicator to somatic cell count for identification of intramammary infection in dairy cows at the end of the lactation period

Daniel Schwarz[a,*,1], Zyncke Lipkens[b,1], Sofie Piepers[b], Sarne De Vliegher[b]

[a] FOSS Analytical A/S, Nils Foss Allé 1, 3400 Hilleroed, Denmark
[b] M-team and Mastitis and Milk Quality Research Unit, Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

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ABSTRACT

The objective of this study was to investigate the new differential somatic cell count (DSCC) as a supplementary indicator to SCC for the identification of intramammary infection (IMI) in dairy cows at the end of the lactation period. Different approaches for identification of cows with IMI (i.e. often based on SCC) and targeted antimicrobial treatment of those rather than of all cows have been developed (i.e. selective dry cow treatment). Recently, DSCC representing the proportion of polymorphonuclear neutrophils and lymphocytes, has been introduced as an additional indicator for the presence of IMI. We used the last dairy herd improvement (DHI) samples taken within 42 d prior to dry-off as well as hand-stripped samples collected within 5 days prior to dry-off to measure DSCC and SCC. The bacteriological status was determined using quarter foremilk samples collected close to drying off. In total, 582 cows were dried off during our study but not all of them could be included in the data analysis for different reasons (e.g. incomplete data, samples too old for reliable determination of SCC and DSCC, contamination). Eventually, the final data set comprised of 310 cows of which 64 and 149 were infected with major and minor pathogens, respectively, and 97 were uninfected. The area under receiver-operating characteristics curves (AUC) were calculated to compare the diagnostic abilities of the different parameters. The AUC for identification of IMI by major pathogens when using the combination of DSCC and SCC was 0.64 compared to 0.62 for SCC alone and 0.62 for DSCC alone. The different parameters were further compared based on test characteristics and predictive values. For example, classifying cows as infected based on a cut-off of 200,000 cells/ml for SCC alone and in terms of using DSCC combined with SCC based on either > 60% and/or > 200,000 cells/ml, the sensitivity changed from 47 to 66% and the specificity from 74 to 54%. At the same time, the negative predictive value changed from 84 to 86% and the positive predictive value from 32 to 27%. Test characteristics and predictive values of the parameters DSCC and SCC were similar using DHI and hand-stripped samples. In conclusion, our study provides first indications on test characteristics and predictive values for the combination of DSCC and SCC. However, more work on this subject and the actual practical application is needed.

1. Introduction

Somatic cell counts (SCC) in milk provide an indication of the inflammatory response in the mammary gland and thus act as a proxy for detecting IMI, also at the end of lactation or dry-off (e.g., Lipkens et al., 2019a). Thus, the SCC results from the milk recording/dairy herd improvement (DHI) testing(s) before dry-off became a main selection criterion as part of the selective dry cow therapy (SDCT) approach, where only those cows most likely to be infected with major pathogens at dry-off are treated with long-acting antimicrobials. Selective dry cow therapy was implemented as such in the Netherlands using the last test-day SCC (Scherpenzeel et al., 2016) without significant impact on udder health yet with an apparent reduction in the use of antimicrobials (Vanhoudt et al., 2018). The latter makes sense as 70–75% of antimicrobials applied on dairy farms are used in relation to udder health, either in form of treatments or in a preventive manner (Stevens et al.,

* Corresponding author.
E-mail address: das@foss.dk (D. Schwarz).
[1] Authors contributed equally.

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2016). Approximately 50% of the total consumption of antimicrobials is used for dry cow treatment (van Werven, 2014).

While SCC represents the total number of cells, cell differentiation refers to the proportions of individual cell populations such as lymphocytes, macrophages, and PMN in milk that play an important role in inflammatory responses within the mammary gland (e.g. Paape et al., 1979). A new parameter for cell differentiation in milk, the differential somatic cell count (DSCC) parameter, has recently been described (Damm et al., 2017) and, similar to SCC, can be determined using dedicated high-throughput flow cytometers for analysis of DHI samples. Briefly, DSCC indicates the percentage of PMN combined with lymphocytes. Proportions of macrophages can be calculated by 100-DSCC. Percentages of DSCC were described to vary broadly in the low SCC range but increase as SCC increases (Damm et al., 2017). In this context, elevated DSCC results are generally assumed to be associated with the presence of IMI (Schwarz et al., 2011a, b; Pilla et al., 2012; Damm et al., 2017). A newly published study indicates that the combination of DSCC and SCC could lead to increased sensitivities in mastitis monitoring through DHI programmes (Wall et al., 2018). More specifically, in a cow with 3 healthy and 1 infected quarters, the infected one would not only be represented by an elevated SCC but also by an elevated DSCC in a cow-composite sample, implying that an individual quarter would be more clearly reflected in cow-composite milk samples.

The objective of our study was to investigate the new DSCC parameter in combination with the well-established SCC as indicator for identification of IMI at the end of the lactation period. This was done by exploring the test characteristics and predictive values of SCC alone, DSCC alone, and, thirdly, using a scenario where both DSCC and SCC were combined. Although DSCC is supposed to be used in combination with SCC (Damm et al., 2018), we have looked at it as a stand-alone parameter as well given the little knowledge on test characteristics and predictive values of DSCC available today.

2. Materials and methods

2.1. Animals and farms

Fifteen dairy farms (in total 1,363 Holstein Friesian cows) in the East and West Flanders provinces, Belgium, with a geometric average bulk tank SCC of ≤250,000 cells/ml in a 6 months period prior to our study were selected. The average herd size was 91 (48 to 215) and average 305 d milk production was at 8,839 kg (ranging from 5,922 to 11,594 kg). Cows were calving year-round. All farms were milking in conventional parlours and applied blanket dry cow therapy.

2.2. Milk sample collection

A total number of 582 cows was dried off on the 15 dairy farms during our study period from April to December. Dairy herd improvement samples were taken as part of the routine milk recording programme carried out by the Dutch/Flemish cattle improvement cooperative CRV (CRV, Arnhem, the Netherlands). A subsample of the DHI sample was collected and used for determination of DSCC and SCC. Only DHI samples from the last DHI testing prior to drying-off were considered (up to 42 d prior to dry-off). Single quarter foremilk samples (volume: 5 ml) were collected aseptically after routine cleaning and disinfection of the udder and discarding of the first streaks of milk within a maximum of 5 d prior to drying-off. At the same milking, from each cow hand-stripped samples (volume: 10 ml) representing an equal (approximately 2.5 ml) amount of milk from each quarter were collected before attaching the milking clusters as well. The quarter foremilk and hand-stripped samples were taken at the same milking and in a period of maximum 5 d prior to drying-off, the DHI sample, however, was collected at a different milking and in a period up to 42 d prior to drying-off. DHI and hand-stripped samples were used for determination of DSCC and SCC, while quarter foremilk samples were used for bacteriological culturing. DHI samples were preserved using Bronopol (BSM Microtabs II, Advanced Instruments, Norwood, USA) and hand-stripped samples were kept at 4^{0}C and processed within 24 h to reflect practical conditions (however, the different types of preservation do not impact DSCC nor SCC results, Damm et al., 2017). Only samples with DSCC and SCC results that were determined within a maximum of 4 d after sample collection and thus according to recommendations (Damm et al., 2017) were included in our study.

2.3. SCC and DSCC analysis

Determination of DSCC and SCC was performed according to Damm et al. (2017). Briefly, milk samples were stained with FOSS DC Reagent (ratio 1:3.2), incubated at 40 °C for 1 min, vortexed for 5 s, and immediately analysed on an Attune (ThermoFisher Scientific, Waltham, USA) flow cytometer. FlowJo (FlowJo LLC, Ashland, USA) software was used to determine DSCC and SCC.

2.4. Bacteriological analysis and definition of intramammary infection status

Bacteriological culturing was performed according to NMC (2004) standards. In brief, 10 µl of milk was streaked onto a quadrant of a blood-aesculin agar plate (Oxoid, Aalst, Belgium) and MacConkey agar plate (Oxoid) and incubated aerobically for 24 to 48 h at 37°C. Identification of bacteria was done by Gram staining and inspection of the colony morphology. Catalase tests were performed to differentiate gram-positive cocci as catalase-positive staphylococci or catalase-negative streptococci. DNAse testing, colony morphology, coagulase testing and haemolysis patterns were used to distinguish *Staphylococcus aureus* from non-*aureus* staphylococci (NAS). Streptococci were subdivided into aesculin-positive streptococci (*Streptococcus uberis* and other aesculin-positive streptococci) and aesculin-negative streptococci (*Streptococcus agalactiae* and *Streptococcus dysgalactiae*) based on the appearance on blood-aesculin and bile-aesculin agar. *Corynebacterium* spp. were differentiated from the catalase-positive staphylococci by colony morphology and Gram staining. *Trueperella pyogenes* was distinguished from the catalase-negative streptococci based on growth characteristics (no growth visible after 24 h of incubation at 37 °C), haemolysis patterns, and Gram staining. Coliforms were considered as *Escherichia (E.) coli* or non *E. coli* Gram negatives based on the appearance on MacConkey's agar. Samples were considered to be culture positive if ≥3 colonies of the same type were observed (≥300 cfu/ml, applied for minor as well as major pathogens) and considered contaminated in case of ≥3 phenotypically different colonies as done elsewhere (Pantoja et al., 2009), with exception of NAS as phenotypically different colonies were allowed because different colony types can belong to the same species (De Visscher et al., 2013).

For determination of the IMI status of the cows the quarter-level bacteriological results were used as follows: 1) A cow was considered to be infected with major pathogens if ≥1 quarters revealed *Staphylococcus (S.) aureus, Streptococcus uberis,* other aesculin-positive streptococci, *Streptococcus dysgalactiae, Trueperella pyogenes* or gram-negative bacteria; 2) A cow was considered to be infected with minor pathogens if ≥1 quarters revealed *Corynebacterium* spp. or NAS and none of the quarters was infected with major pathogens; 3) A cow was considered non-infected only in case of culture-negative or non-significant growth results of all four quarters. Cows with detection of both minor and major pathogens were considered as infected with major pathogens.

2.5. Data set

582 cows were dried off during our study period and, in total, 200 cows had to be excluded from our dataset because of different reasons. Specifically, 67 cows were excluded due to incomplete data [no DHI

Table 1
Prevalence and distribution of mastitis pathogens isolated from 310 cows and 1,240 quarters from 15 dairy herds in Flanders. Quarter foremilk samples were collected within 5 d prior to drying-off and analysed using bacteriological culture.

	Cow level			Quarter level		
Pathogen	n	% of total[1]	% of infected by either major or minor pathogen	n	% of total[2]	% of infected by either major or minor pathogen
None	97	31.29		733	59.11	
Major pathogens	64	20.65		66	5.32	
Staphylococcus aureus	4	1.29	1.88	4	0.32	0.81
Streptococcus uberis	24	7.74	11.27	25	2.02	5.06
other aesculin-positive streptococci	19	6.13	8.92	19	1.53	3.85
Streptococcus dysgalactiae	9	2.90	4.23	10	0.81	2.02
Escherichia coli	4	1.29	1.88	4	0.32	0.81
Others[3]	4	1.29	1.88	4	0.32	0.81
Minor pathogens	149	48.06		428	34.52	0.00
NAS[4]	67	21.61	31.46	184	14.84	37.25
Corynebacterium species	64	20.65	30.05	236	19.03	47.77
NAS[4] and *Corynebacterium species*	18	5.81	8.45	8	0.65	1.62

[1] Percentage of all cows (n = 310).
[2] Percentage of all quarters (n = 1,240), 13 quarters were blind.
[3] Others: *Pseudomonas* spp., *Trueperella pyogenes*.
[4] Non-*aureus* staphylococci.

sampling prior to drying-off (n = 25), no hand-stripped sample (n = 35), not sampled for culturing (n = 5) or sample collected too early (i.e. > 5 d before drying-off, n = 2)]. In case of 95 cows either the DHI sample or the hand-stripped sample was too old (> 4 d) for reliable determination of DSCC and SCC. A further 38 cows were removed from the data set because of incomplete DHI data (e.g. mismatch of cow and sample ID and thus absence of parity information).

Bacteriological results were available for a total number of 382 cows. However, in case of 72 cows the IMI status was undefined [i.e., detection of *Bacillus* spp. (n = 19) or contamination (n = 53) in at least one quarter] and thus redundant for further analyses.

In terms of DSCC, results in the range below 50,000 cells/ml are not reliable (e.g. poor repeatability) because they are out of the performance range of the method as described elsewhere (Damm et al., 2017). To be able to keep samples with SCC results in that range in the data set, DSCC results were set to 45% (meaning 45% of somatic cells are PMN and lymphocytes and 55% macrophages) as done previously (Wall et al., 2018). A predominance of macrophages in low SCC samples has been described in different studies (Ostensson et al., 1988). Ninety-five DHI samples and 102 hand-stripped samples were affected, respectively.

2.6. Statistical analysis

The parameters DSCC alone and SCC alone were treated separately and analysed at cow level.

Factors affecting DSCC and SCC. Somatic cell count was normalised using a natural log transformation whereas DSCC was normalised using asin(sqrt(p)) with p = DSCC%/100, ranging from 0 to 1. The data was analysed using the lme package in R, version 3.5.1 (The R Foundation, Vienna, Austria), and the following statistical model:

$$Y_{ijk} = \mu + IMI_{j(k)} + P_{j(k)} + T_{j(k)} + Hj + e_{ijk}$$

where Y_{ijk} = observed value for DSCC or SCC of cow i in herd j; μ = overall mean; IMI $j_{(k)}$ = fixed effect of intramammary infection status (no, minor, or major pathogens); P $j_{(k)}$ = fixed effect of parity (1, 2, 3, 4+), T $j_{(k)}$ = fixed effect of time of collection prior to dry-off (< 14 vs. ≥ 14 d) (only used for modelling results of DHI samples but not hand-stripped samples); H_k = random effect of herd (1–15) correcting for potential clustering of cows within herds; and e_{ijk} = random error term. Breed and DIM were not included in the models because all cows included in the study were Holstein Friesian and at the end of lactation (mean ± SE: 333 ± 4 d). Estimated marginal means (EMM/least-square means) of each parameter (SCC EMM were back transformed to cells/ml, no back transformation for DSCC) were computed for each pathogen group and compared using the Tukey test.

Analysis of test characteristics and predictive values. Sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) for detection of IMI with major pathogens (as treatment of IMI by minor pathogens is unwanted) at the end of lactation were calculated including 95% confidence intervals each. The parameters SCC alone, DSCC alone, and the combination of DSCC and SCC were evaluated based on exemplary cut-offs. Results were calculated separately for DHI and hand-stripped samples. In case of evaluating the combination of DSCC and SCC for the diagnosis of IMI by major pathogens, cows were considered infected if any (or both) of the measurements was above the chosen cut-off.

Receiver-operating characteristics analysis. Receiver-operating characteristics (ROC) analysis was performed and results were compared between the different parameters using the pROC package (Robin et al., 2011). Optimal cut-offs were determined for each parameter using the OptimalCutpoints-Package (Lopez-Raton et al., 2014) and the maximise Se and Sp method.

3. Results

3.1. Intramammary infection statuses, DSCC, and SCC

At the quarter level, 733 quarters were uninfected, 66 revealed an IMI by major pathogens (i.e. *E. coli*: 4, *Streptococcus uberis*: 25, other aesculin-positive streptococci: 19, *S. aureus*: 4, *Streptococcus dysgalactiae*: 10, others: 4), 428 revealed IMI by minor pathogens (i.e. NAS: 184, *Corynebacterium* spp.: 236, NAS and *Corynebacterium* spp.: 8) and 13 quarters were blind (Table 1). In total, DSCC and SCC as well as bacteriological results were available from 310 cows. While 97 cows were considered uninfected (Table 1), 149 were infected with minor pathogens (i.e. NAS: 67, *Corynebacterium* spp.: 64, NAS and *Corynebacterium* spp.: 18). Major pathogens (i.e. *E. coli*: 4, *Streptococcus uberis*: 24, other aesculin-positive streptococci: 19, *S. aureus*: 4, *Streptococcus dysgalactiae*: 9, others: 4) were found in 64 cows.

Differential SCC results in DHI samples generally varied broadly in the range 50,000–400,000 cells/ml and tended to vary in a narrower band but at a higher level at higher SCC (Fig. 1). The majority of the samples (i.e. 43 out of 64) with detection of major pathogens appeared in the range DSCC > 50%. Differential SCC as well as SCC results varied widely (26–87%, 12,000–3,315,000 cells/ml) in samples with detection of minor pathogens. Results of uninfected cows occurred mainly in the range below 400,000 cells/ml and showed widely varying DSCC values (20–82%).

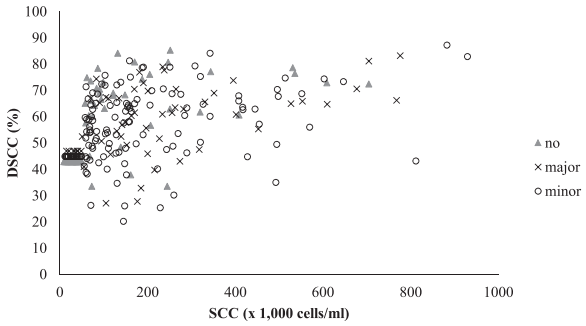

Fig. 1. Differential somatic cell count (DSCC) versus SCC results in DHI samples taken during last milk recording within 42 d prior to dry-off according to pathogens detected: (▲) no (n = 97), minor (n = 149), (×) major pathogens (n = 64). Each symbol represents the results of one DHI sample/cow but overlapping is possible.

Differential SCC in DHI samples was associated ($P < 0.001$) with the cow-level IMI status but not with parity or time of collection prior to dry-off (< 14 vs. ≥14 d). Somatic cell count in DHI samples was associated ($P < 0.001$) with the cow-level IMI status as well as with parity. The associations described above were the same in hand-stripped samples and DHI samples for both parameters, except that time of collection prior to dry-off was not tested with hand-stripped samples.

As for DHI samples, estimated marginal mean (EMM) DSCC results of cows infected with minor pathogens (EMM ± SE: 0.88 ± 0.02) or major pathogens (0.90 ± 0.02) were significantly ($P < 0.01$) higher than those in uninfected cows (0.78 ± 0.02). The difference of the EMM DSCC was not statistically significant ($P > 0.05$) between cows infected with minor pathogens and those infected with major pathogens. Estimated marginal mean SCC results were evidently ($P < 0.01$) higher in cows with IMI by major pathogens (183,637 ± 13,642 cells/ml) compared to cows infected with minor pathogens (152,662 ± 11,087 cells/ml) or non-infected cows (81,633 ± 13,274 cells/ml).

Differential SCC results in hand-stripped samples followed the same pattern as in DHI samples (non-infected: 0.78 ± 0.02, minor: 0.83 ± 0.01, major: 0.86 ± 0.02) and results were significantly ($P < 0.001$) higher in cows infected with minor and major pathogens compared to non-infected cows. Differences between the cows infected by minor or major pathogens were not significant ($P > 0.05$). Estimated marginal mean SCC results were clearly ($P < 0.05$) lower in non-infected cows (76,733 ± 12,764 cells/ml) and those infected by minor pathogens (154,540 ± 12,144 cells/ml) compared to cows with IMI by major pathogens (191,202 ± 13,787 cells/ml).

3.2. Test characteristics and predictive values of DSCC, SCC, and DSCC and SCC combined

Sensitivity (Se), Sp, PPV, and NPV for detection of IMI with major pathogens at the end of lactation based on either DHI or hand-stripped samples were calculated with 95% confidence intervals for SCC alone, DSCC alone, and the combination of DSCC and SCC (Table 2). As exemplary cut-offs, 100,000 and 200,000 cells/ml were evaluated using SCC alone and 65.63% and 46.88% out of 64 cows with major pathogen infections were identified correctly, respectively, working with DHI samples (Table 2 for Se, Sp, PPV, NPV). In terms of DSCC alone, the exemplary cut-offs 50, 60 and 70% were assessed and led to correct identification of 67.19, 57.81, and 31.25% of cows with major pathogen infections, respectively. The combination of DSCC and SCC resulted in correct identification of 78.13, 71.88, and 70.31% of cows using 100,000 cells/ml and 50, 60 and 70%, respectively, as exemplary cut-offs. A SCC threshold of 200,000 cells/ml in combination with DSCC cut-offs of 50, 60 and 70% enabled correct identification of 73.44, 65.63, and 54.69% of cows with major pathogen infections, respectively. The test characteristics and predictive values were at comparable levels for SCC alone and DSCC alone. The combination of DSCC and SCC led to slightly higher Se and NPV results but slightly lower Sp and PPV results compared to SCC alone. However, the 95% confidence intervals (CI) of the test characteristics and predictive values were overlapping for all parameters and exemplary cut-offs (Table 2). In case of comparing SCC alone (cut-off of 200,000 cells/ml) with the combination of DSCC and SCC (cut-offs of 200,000 cells/ml and 50%), 95% CI of Se and Sp were not overlapping. The diagnostic abilities of SCC alone, DSCC alone, and the combination of DSCC and SCC were similar for DHI and hand-stripped samples (Table 2).

Receiver operating characteristics analysis

The area under a ROC curve (AUC) quantifies the overall ability of a test to discriminate between healthy and infected individuals and was calculated to compare the different parameters used for identification of IMI (i.e. by major pathogens only) at dry-off in this study. The AUC for the combination of DSCC and SCC was 0.64 (95% CI: 0.56–0.72) and not significantly ($P > 0.05$, DeLong's test) different from the AUC of SCC alone (0.62, 95% CI: 0.55-0.70) or DSCC alone (0.62, 95% CI: 0.55–0.70). The optimal cut-offs for SCC alone, DSCC alone, and the combination of both using DHI samples were 131,000 cells/ml, 59%, and 135,000 cells/ml and 55%, respectively. In terms of hand-stripped samples, AUC was also 0.64 (95% CI: 0.55–0.71) for the combination of DSCC and SCC and not significantly ($P > 0.05$) different from the AUC of SCC alone (0.62, 95% CI: 0.55-0.71) or DSCC alone (0.58, 95% CI: 0.50–0.66).

4. Discussion

The bulk of antimicrobials applied on dairy farms is used in connection with blanket dry cow therapy, typically regardless of the actual IMI status. Approaches to reduce the amount of antimicrobials consumed (i.e. SDCT) can be based on culture (e.g. Patel et al., 2017) or PCR (e.g. Gussmann et al., 2018) but the tests are costly and time-consuming (e.g. specific samples needed (e.g. sterile), labour time, materials). Besides, SCC results from monthly available milk recording samples are broadly used as practical and convenient key indicator for SDCT in some countries nowadays (e.g. Scherpenzeel et al., 2016; Vanhoudt et al., 2018; Vilar et al., 2018). Recently, DSCC, a new parameter that can routinely be determined on dedicated high-throughput flow cytometers in connection with SCC, was introduced (Damm et al., 2017). Hence, the objective of our study was to investigate DSCC in combination with the well-established SCC as indicator for identification of IMI at the end of the lactation period.

Although a total number of 582 cows was dried-off in our study, we were only able to use results of 310 cows for investigating DSCC as a supplementary indicator to SCC. This was mainly caused by missing or unreliable data and contaminated milk samples, as described above. Unfortunately, it was not possible for us to optimise the sampling and testing procedures while collecting data. Nevertheless, the prevalence and distribution of mastitis pathogens found in our study is comparable to previous observations in Flanders, Belgium, (Piepers et al., 2007) or Germany (Schwarz et al., 2010). We further noticed that the cows removed from the data set were from all different farms rather than from specific farms only. Hence, we concluded that our data set is still representative.

The FOSS DSCC method does not allow to generate reliable DSCC data in the range < 50,000 cells/ml given the low numbers of cells available to actually determine DSCC as described in detail elsewhere (Damm et al., 2017). To be able to work with data in that SCC range though, we decided to set the DSCC result to 45% as done elsewhere (Wall et al., 2018). This indicates a predominance of macrophages in low SCC samples, which has been described before (Ostensson et al., 1988).

To the best of our knowledge, this is the first study describing DSCC in connection with identification of IMI at the end of lactation.

Table 2
Results (%) for sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) including 95% confidence intervals (CI) for detection of IMI with major pathogens using DHI and hand-stripped samples collected prior to dry-off, respectively, when applying different cut-offs of SCC (cells/ml) alone, DSCC (%) alone, and a combination of SCC and DSCC.

Item	Cut-off(s)	Correctly identified, n (%)		Se (95%CI)	Sp (95%CI)	PPV (95%CI)	NPV (95%CI)
		with IMI	without IMI				
DHI samples							
SCC (cells/ml)	100,000	42 (65.63)	142 (50.81)	65.63 (53.99 77.26)	50.81 (44.57 57.06)	25.77 (19.05 32.48)	85.03 (79.27 90.80)
	200,000	30 (46.88)	182 (73.98)	46.88 (34.65 59.10)	73.98 (68.50 79.47)	31.91 (22.49 41.34)	84.26 (79.40 89.12)
DSCC (%)	50	43 (67.19)	120 (48.78)	67.19 (55.68 78.69)	48.78 (42.53 55.03)	25.44 (18.88 32.01)	85.11 (79.23 90.98)
	60	37 (57.81)	151 (61.38)	57.81 (45.71 69.91)	61.38 (55.30 67.47)	28.03 (20.37 35.69)	84.83 (79.56 90.10)
	70	20 (31.25)	203 (82.52)	31.25 (19.89 42.61)	82.52 (77.77 87.27)	31.75 (20.25 43.24)	82.19 (77.41 86.96)
SCC (cells/ml) and DSCC (%)	100,000; 50	50 (78.13)	92 (37.40)	78.13 (68.00 88.25)	37.40 (31.35 43.44)	24.51 (18.61 30.41)	86.79 (80.35 93.24)
	100,000; 60	46 (71.88)	104 (42.28)	71.88 (60.86 82.89)	42.28 (36.10 48.45)	24.47 (18.32 30.61)	85.25 (78.95 91.54)
	100,000; 70	45 (70.31)	119 (48.57)	70.31 (59.12 81.51)	48.57 (42.31 54.83)	26.32 (19.72 32.92)	86.23 (80.48 91.98)
	200,000; 50	47 (73.44)	109 (44.31)	73.44 (62.62 84.26)	44.31 (38.10 50.52)	25.54 (19.24 31.84)	86.51 (80.54 92.47)
	200,000; 60	42 (65.63)	134 (54.47)	65.63 (53.99 77.26)	54.47 (48.25 60.69)	27.27 (20.24 24.31)	85.90 (80.44 91.96)
	200,000; 70	35 (54.69)	164 (66.94)	54.69 (42.49 66.88)	66.94 (61.05 72.83)	30.17 (21.82 38.53)	84.97 (79.93 90.02)
Hand-stripped samples							
SCC (cells/ml)	100,000	43 (67.19)	129 (52.44)	67.19 (55.68 78.69)	52.44 (46.20 58.68)	26.88 (20.01 33.74)	86.00 (80.45 91.55)
	200,000	30 (46.88)	172 (69.92)	46.88 (34.65 59.10)	69.92 (64.19 75.65)	28.85 (20.14 37.55)	83.50 (78.43 88.56)
DSCC (%)	50	35 (54.69)	138 (56.10)	54.69 (42.49 66.88)	56.10 (49.90 62.30)	24.48 (17.43 31.52)	82.63 (76.89 88.38)
	60	22 (34.38)	184 (74.80)	34.38 (22.74 46.01)	74.80 (69.37 80.22)	26.19 (16.79 35.59)	81.42 (76.34 86.49)
	70	7 (10.94)	224 (91.06)	10.94 (3.29 18.58)	91.06 (87.49 94.62)	24.14 (8.56 39.71)	79.72 (75.01 84.42)
SCC cells/ml) and DSCC (%)	100,000; 50	49 (76.56)	101 (41.87)	76.56 (66.18 86.94)	41.87 (35.70 48.04)	24.74 (18.60 30.87)	85.83 (79.59 92.07)
	100,000; 60	47 (73.44)	117 (47.56)	73.44 (62.62 84.26)	47.56(41.32 53.80)	26.70 (20.17 33.24)	87.31 (81.68 92.95)
	100,000; 70	45 (70.31)	125 (51.63)	70.31 (59.12 81.51)	51.63 (45.38 57.87)	27.44 (20.61 34.27)	86.99 (81.53 92.44)
	200,000; 50	44 (68.75)	116 (48.98)	68.75 (56.49 79.57)	48.98 (42.72 55.24)	25.60 (19.00 32.19)	85.11 (79.23 90.98)
	200,000; 60	38 (59.38)	146 (59.59)	59.38 (47.34 71.41)	59.59 (53.45 65.74)	27.74 (20.24 25.23)	84.88 (79.53 90.24)
	200,000; 70	33 (51.56)	168 (68.29)	51.56 (39.32 63.81)	68.29 (62.48 74.11)	29.73 (21.23 38.23)	84.42 (79.38 89.46)

However, we observed a similar pattern in the SCC vs. DSCC plot (Fig. 1) as described by others previously (Damm et al., 2017; Wall et al., 2018). Our data showed that the new DSCC parameter was significantly higher in cows with IMI by major pathogens at drying off compared to uninfected cows. Given that DSCC mainly represents the proportion of PMN (Damm et al., 2017) and the fact that PMN are known to be the dominant cell population in the presence of major pathogens (e.g. Paape et al., 1979; Schwarz et al., 2011a; Pilla et al., 2012), these results were to be expected. Interestingly, IMI by minor pathogens caused significantly higher DSCC and SCC results compared to no IMI but there was no significant difference compared to IMI by major pathogens. Generally, huge variations in both DSCC and SCC results were seen in presence of IMI by minor pathogens. This might be explainable by the varying pathogenicity of particularly NAS species and strains (De Vliegher et al., 2012; Vanderhaeghen et al., 2015), where some species are known to be less or more relevant for udder health than others, and is further discussed below.

The test characteristics of DSCC and SCC, individually and combined, was evaluated based on comparing Se, Sp, PPV, and NPV as well as AUC analyses quantifying the overall ability of a test to discriminate between healthy and diseased individuals. Comparable data on DSCC does not exists in the literature given the novelty of the parameter. However, Nyman et al. (2016) describes AUC results of 0.83 for SCC as indicator of mastitis during lactation. In a study comparable to ours but investigating only SCC in DHI samples as indicator for SDCT, an AUC of 0.85 is reported (McDougall et al., 2017). However, the definition of IMI is critical when comparing results of different studies. While we defined the IMI status of a cow based on 1 sampling and detection of ≥ 3 colonies of the same type of pathogen, Nyman et al. (2016) used 3 consecutive samples whereas McDougall et al. (2017) did not specify details of the bacteriological test performed (e.g. detection limit used). Area under the curve results in our study are generally fairly low which might be explained by the fact that 14 and 6 cows with major pathogens infection had $<$ 50,000 cells/ml in DHI and hand-stripped samples, each. At the same time, 74 and 82 cows with no or minor pathogen results showed $>$ 200,000 cells/ml in DHI and hand-stripped samples each. This, in turn, caused evident overlapping of test positive and negative results. A possible reason might be misclassification of the infection statuses of cows. It is well-known that pathogens are present in the udder but negative culture results occur due to intermittent shedding (e.g. *S. aureus*), shedding of too-low masses of a pathogen to grow during bacteriological culture and ceased growth of pathogens (Sears et al., 1990). Presence of major pathogens in samples with low SCC, however, has been described in other studies (e.g. Schwarz et al., 2010) and were interpreted as mastitis in its early stage because of elevated proportions of PMN confirming inflammatory reactions (Schwarz et al., 2011a, b). Nevertheless, SCC is generally considered as highly valuable for dry-off treatment decision making (Rajala-Schultz et al., 2011; Dufour and Dohoo, 2012; Scherpenzeel et al., 2014; Lipkens et al., 2019a) and used widely in practice for that purpose.

Cows infected with minor pathogens were regarded suspicious but not considered as worth treating with antimicrobials in our study, which is in line with common practise elsewhere (e.g. Østeras and Sølverød, 2009). Thus, such cows were grouped together with non-infected cows for ROC analyses and calculations of test characteristics and predictive values. However, both DSCC and SCC results of such cows varied hugely. While *Corynebacterium* spp. is known to readily colonize the teat canal (Brooks and Barnum, 1984), De Vliegher et al. (2012) described that the pathogenicity of NAS varies from being protective to being the cause of subclinical mastitis or (mild) clinical mastitis. Worldwide, research is ongoing to further explore the pathogenicity of the different NAS species. Intramammary infection by the more harmful NAS such as *S. chromogenes*, *S. simulans*, and *S. xylosus* (Supré et al., 2011) typically results in somewhat higher SCC. In this context, the new DSCC parameter might actually open up the possibility to further differentiate between less and more harmful NAS species by indicating the proportions of PMN, which, in turn, are a clear indicator of an inflammatory reaction even in the low SCC range (e.g. Schwarz et al., 2011a, b). Polymorphonuclear neutrophil proportions in quarters with detection of minor pathogens were described to be significantly higher compared to those in non-infected quarters (Schwarz et al., 2011a; Pilla et al., 2012). As a result, it could be hypothesised that elevated DSCC

results might indicate more harmful minor pathogens needing more attention. In that event, the number of false-positives would be clearly lower in our study. However, species information on NAS was not available in our study and further research is needed to better understand the relation between DSCC and IMI by NAS.

We used and evaluated the diagnostic abilities of the last DHI sample collected within a period of up to 42 d as well as hand-stripped samples collected within 5 d before dry-off. It is generally known that quarter samples allow a more precise determination of the udder health status than cow-composite samples due to the so-called dilution effect (e.g. Reyher and Dohoo, 2011; Dufour and Dohoo, 2012). However, we deliberately choose to work with cow-composite samples because, in particular, such samples are routinely available in practice anyway. In our study, the AUC results for the different parameters investigated were at the same level in DHI and hand-stripped samples. Hence, the readily available DHI samples from typically monthly testing in the frame of milk recording programmes could be used and the additional effort and expenses required for collection and analysis of hand-stripped samples would not be justified. To the best of our knowledge, this is the first study investigating DSCC in the context of mastitis management, but using SCC results of the last DHI sample before dry-off has been described as a reliable predictor of IMI at dry-off previously (McDougall et al., 2017). The fact that AUC results for the different parameters tested were at the same levels for DHI and hand-stripped samples further clearly opens up a highly interesting possibility for dairy farms not enrolled to regular DHI testing. Hand-stripped samples of cows approaching the dry period could be collected and analysed and thus be used to decide about (selective) dry cow therapy.

In our study, single testing of DHI and hand-stripped samples was performed due to practical reasons. It has been demonstrated that multiple (i.e. triplicate) tests lead to a modest gain in Sp but little or no gain in Se (Dohoo et al., 2011) in detecting IMI. Lipkens et al. (2019a) further demonstrated that using SCC from multiple test-days as well as other information such as prevalence of subclinical mastitis in the herd can help to better detect uninfected cows at the end of lactation.

The identification of as many IMI caused by major pathogens as possible is clearly desired in terms of SDCT because such cases are clearly a risk for udder health issues at the beginning of the subsequent lactation when undetected and left untreated at dry-off (Lipkens et al., 2019b). At the same time, antimicrobial treatment should only be withheld from truly uninfected cows, to maximally protect udder health. Hence, parameters and cut-offs with the highest Se and NPV would be the best choice.

5. Conclusions

This is a first study investigating the new DSCC parameter as a supplementary indicator to SCC for identification of IMI at the end of the lactation period. Although 582 cows were dried off during our study period, not more than 310 cows were included in the final data analysis because of missing or unreliable data and contaminated milk samples. However, our data is still considered representative and reveals that the combination of DSCC and SCC helps to improve Se and NPV yet at the same time Sp and PPV decrease. We further found that the test characteristics for hand-stripped samples collected up to 5 d prior to dry off were similar to those of the last DHI sample collected up to 42 d to dry-off and thus either type of sample could be used in practise. However, more work is needed to advance the actual practical application of the combination of DSCC and SCC in connection with identification of IMI at the end of the lactation period.

The first author of this publication is employed with FOSS Analytical A/S, the entity manufacturing and selling Fossomatic 7 DC (among other products). The co-authors are working with Ghent University and have no conflicts of interest.

Declaration of Competing Interest

The first author of this publication is employed with FOSS Analytical A/S, the entity manufacturing and selling Fossomatic 7 DC (among other products). The co-authors are working with Ghent University and have no conflicts of interest.

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