Differential Somatic Cell Count (DSCC) – a rationale for the new parameter

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It is well-known that not only the total somatic cell count (SCC), but also the composition of somatic cells changes significantly during the process of mastitis. Specifically, SCC in milk from healthy udder quarters is low and consists mainly of macrophages and lymphocytes (Lee et al., 1980; Schwarz et al., 2011a; b; Pilla et al., 2012). In milk of an infected mammary gland, however, high total numbers of cells being mainly polymorphonuclear neutrophils (PMN) can be found (Paape et al., 2002). These changes in the composition are explainable by the respective functions of individual immune cells (Sordillo et al., 1997; Oviedo-Boyso et al., 2007): Lymphocytes regulate the induction and suppression of immune responses. Macrophages recognize invading mastitis pathogens and initiate the immune response by starting a massive influx of PMN. Beyond that, macrophages ingest bacteria, cellular debris, and accumulated milk components and carry out tissue repair. PMN cells defend against invading bacteria at the beginning of an infection.

Scientific literature concludes that besides the determination of the total SCC, differentiation of cells gives valuable additional information for a more precise description of the actual udder health status of dairy cows (Pillai et al., 2001; Rivas et al., 2001; Pilla et al., 2013).

With the new Fossomatic™ 7 DC, a novel milk testing parameter, Differential Somatic Cell Count (DSCC), for enhanced mastitis screening and management is introduced. The DSCC represents the combined proportion of PMN and lymphocytes in percent. The percentage of macrophages is 100 – DSCC. The combination of PMN and lymphocytes into one group is explained in the following.

Differentiation of cells in DHI samples

The Fossomatic 7 DC will mainly be applied in central milk testing laboratories analysing individual cow milk samples for dairy herd improvement (DHI) testing. Hence, 223 routinely available DHI samples were investigated in terms of the distribution of the cell populations lymphocytes, macrophages and PMN. In this context, fluorescence microscopy (the FOSS internal reference method for DSCC) was applied allowing a differentiation of cells into all three cell populations.

SCC of the samples used covered a wide range from 20,000 to 4,218,000 cells/ml (Figure 1). Lymphocytes were generally found with low proportions (0 to 19%) across the entire SCC range and did not indicate any correlation to SCC (correlation coefficient, r = -0.1851). On the contrary, proportions of both macrophages and PMN clearly varied depending on SCC. Macrophages were found with proportions from 10 to 73% and indicated a decrease as SCC increased (r = -0.3172). Proportions of PMN appeared in an even wider range from 13 to 90% and an increase, as SCC increased, was found (r = 0.3307).

There were two reasons for the combination of lymphocytes and PMN into one group: Firstly and most importantly, only two of the three cell populations occurring in milk showed clear variations depending on SCC and thus the actual health status of the mammary gland. Our studies revealed that proportions of macrophages decreased while those of PMN increased as SCC increased. Lymphocytes, however, were found with consistently low proportions across the entire SCC range. This observation is not in line with recent cell differentiation studies (Schwarz et al., 2011a; b, Pilla et al., 2012, 2013), where proportions of macrophages were found to be fairly consistent while those of lymphocytes and PMN developed differently as SCC increased. The different observations

might be explainable by the fact that evidently different methods for differentiation of cells were applied. The FOSS DSCC method is based on the analysis of untreated DHI samples, whereas various centrifugation and washing steps, that can actually alter the composition of cells (Schröder and Hamann, 2005), were required in literature studies. Moreover, the type of milk samples clearly differed: metered DHI samples, representing a representative sample of the entire milking procedure of a cow, were used in FOSS studies, whereas quarter foremilk samples were collected in literature studies. It is well-known that both SCC and the composition of cells in milk differ during the milking procedure (Sarikaya et al., 2005). Nevertheless, the increase of PMN as SCC increase observed in our studies is in accordance with findings in the literature (e.g. Kehrli and Shuster, 1994).

Secondly, the Fossomatic 7 DC is supposed to analyse 600 samples per hour, which allows just six seconds of analysis per sample. This sets limitations on the amount of information that can be gathered per cell, which obviously depends on the staining and treatment of cells as well as the flow cytometric system for reading cell signals.
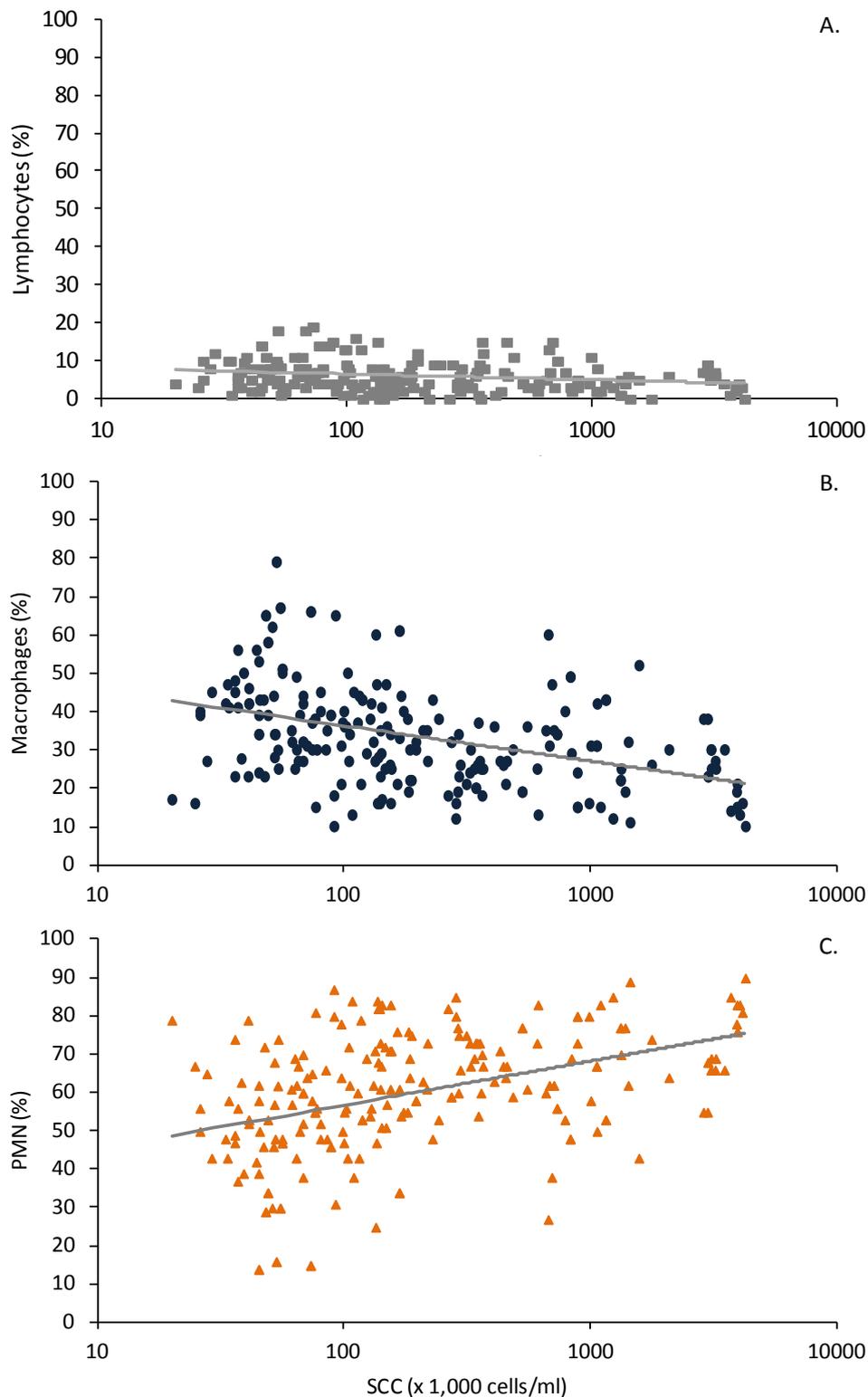
Figure 1. Proportions of a) lymphocytes, b) macrophages and c) polymorphonuclear neutrophil (PMN) determined by the FOSS DSCC method applied on a fluorescence microscope depending on SCC. In total, 223 routinely available DHI samples were analysed. Data is illustrated in combination with linear trendlines. Each symbol represents the result of one DHI sample, but overlapping is possible.

The new DSCC parameter

The relation between the new DSCC parameter and SCC was further investigated by testing routinely collected DHI samples available from a dairy farm with 188 Holstein Frisian cows. SCC in the samples tested ranged between 14,000 and 1,119,000 cells/ml (Figure 2). DSCC values varied broadly from 34% to 79% in the range <400,000 cells/ml. Samples with higher SCC revealed DSCC values at a higher level from 53 to 89%. DSCC values tended to increase as SCC values increase.

The general understanding of researchers (e.g., Lee et al., 1980; Östensson et al., 1988) is that percentages of macrophages in milk are predominant when the mammary gland is uninfected, which is in line with our observations given that numerous samples revealed DSCC values <50% in the SCC range <200,000 cells/ml. More interestingly, a significant number of samples with <200,000 cells/ml revealed DSCC >70%. Elevated proportions of PMN were found previously (Schwarz et al., 2011a; b; Pilla et al., 2012) and interpreted as early indication of intramammary infections, despite unsuspiciously low SCC.

Elevated proportions of PMN in high SCC samples are generally understood as a clear sign of infection (Sordillo et al., 1997; Oviedo-Boyso et al., 2007). Low proportions of DSCC in the high SCC range could be an indication for chronic mastitis cases, given that predominating proportions of macrophages were detected in chronically infected cows previously (Leitner et al., 2000). However, more research is needed to understand the practical application of DSCC in the frame of DHI testing.
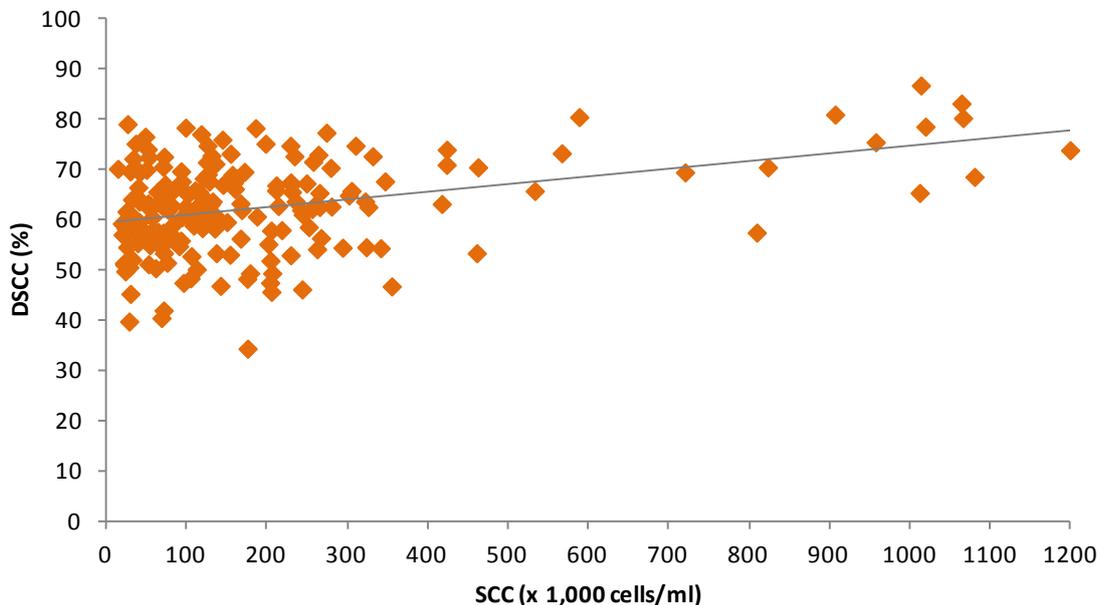

Figure 2. SCC vs. DSCC results from 188 DHI samples originating from one dairy farm. Data is illustrated in combination with a linear trendline. Each symbol represents the result of one DHI sample, but overlapping is possible.

Conclusions

The new DSCC parameter represents the combined proportion of PMN and lymphocytes in percent. The proportion of macrophages is 100-DSCC. PMN and lymphocytes were combined into one group because proportions of lymphocytes in DHI samples turned out to be constantly low and independent from the actual health status of the mammary gland. Instead, macrophages and PMN indicated an antidromic development as SCC increased. Hence, DSCC values increase with increased signs of mastitis.

With the Fossomatic 7 DC two parameters, SCC and DSCC, can be measured simultaneously at low cost. The combination of SCC and DSCC provides more precise information on the actual inflammatory status of the mammary gland. However, the actual application of the two parameters in routine mastitis screening and management programmes carried out within DHI testing requires further research.

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