

## Additional value of cell differentiation in the course of DHI testing

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As a well-established parameter in monthly DHI testing, somatic cell count (SCC) is being used to monitor udder health and to support management decisions on dairy farms.

### Abstract

The aim of this study was to evaluate, whether the additional analysis of cell differentiation in the course of DHI testing could further enhance the informative value of DHI results, e.g. in the form of prognostic key figures for udder health. Using a new generation of high throughput devices, SCC and cell differentiation index (CDI) were analysed simultaneously. The CDI essentially reflects the proportion of macrophages in the total SCC. Cell differentiation was routinely performed from DHI samples taken over a period of 1.5 years from approximately 920,000 animals, partly from robot farms in two different German federal states. Additionally, an experiment including 2,500 animals was conducted over a period of up to 6 months: DHI samples were analysed with regard to SCC and CDI. Simultaneously, SCC, CDI, and the bacteriological status were assessed from udder quarter level samples of the same animals. The data set was supplemented by additional information in regard to diagnosis and treatment of animals.

Statistical analysis of the collected data reveals a complex interaction of cell count and CDI, making it difficult to directly generate additional value from cell differentiation separately from somatic cell count results. Furthermore, it is impractical to model acute inflammatory processes of the udder due to the common interval of four weeks in between DHI testing dates. Nevertheless, two statistical models including CDI and additional DHI parameters could be established in order to predict cell counts in the future. In the case of currently > 100,000 cells/ml, the probability for elevated cell counts in the next two months can be predicted. Whereas in the case of currently < 100,000 cells/ml, the probability for stable udder health with low cell counts in the next two months is predicted. By providing the probability for different outcome scenarios, farmers would be able to rank their animals according to high or low risk and prioritize their effort. The data from quarter milk samples including the bacteriological status is currently being evaluated and preliminary results will be available soon. They will serve as reference to DHI samples and give more detailed insight into actual processes in the udder and the potential further value of CDI.

*Keywords: udder health, DHI data, cell differentiation, somatic cell count, statistical models, prognostic key figures, bacteriological status.*

## Introduction

Mastitis is still one of the most common diseases in dairy farms, influencing not only animal welfare adversely but also the economic performance. Checking the somatic cell count (SCC) routinely in the course of DHI testing is the best way to indirectly assess the current udder health status of cows. Additional knowledge on how udder health is likely to develop in the future will be beneficial to farmers in order to establish even more effective udder health monitoring.

Differential somatic cell count in milk is described as a method to identify intramammary processes in the udder more precisely (Pilla *et al.*, 2013). The aim of the German ZellDiX project is to enhance the informative value of DHI results by evaluating the additional value of differential somatic cell count (DSCC) and by establishing statistical models that reliably predict future udder health.

## Material and methods

Since the introduction of a new generation of devices (Fossomatic 7 DC, FOSS, Denmark) (Damm *et al.*, 2017), not only the total SCC, but also the differential somatic cell count (DSCC) can be analysed routinely in a high throughput manner allowing the assessment of the cell differentiation index (CDI). The CDI essentially reflects the proportion of macrophages in the total SCC. DHI data sets of 920,000 animals were routinely obtained on 19,000 different farms in Germany over consecutive months. The data set consisted of highly diverse farms in respect to size and management type. In the first step of the data analysis we fitted two statistical models, one for chronic SCC elevations and one for stable good udder health. Chronic elevations were defined as cell counts above a defined threshold in the next two DHI measurements. In order to meet different farmers' needs, results were derived for SCC thresholds between 200,000 and 700,000 cells/ml. Cows with stable good udder health had SCC values below 100,000 cells/ml in the next two DHI measurements. For mathematical modelling, we used generalized additive models (GAM) (Wood, 2008) with penalized cubic regression splines. Both models were 10-fold cross-validated and tested using internal and external validation data. In addition to these predictions, GAMs allowed us to identify biases in the underlying data set and the impacts of individual parameters.

In an initial step, a descriptive analysis of the data pool was done in order to characterize the correlation of cell count and CDI using the software "R" (Version 3.52, R Foundation Vienna). Heat maps were used to visually describe the probability for a cell count increase in the next month in relation to CDI and cell count in the initial month (Figure 1).

In a second step, models were established in order to predict individual risks for stable udder health (Figure 2) and for chronic udder impairment considering different initial SCC values (Figure 3). The additional value of CDI was evaluated for SCC-only models and full models (Figure 4).

## Results

The descriptive analysis shows, that for animals with an already elevated current cell count above 200,000 SCC/ml the combination of cell count and CDI has good potential for predicting a sustained cell count elevation (next 2 months above 200,000 SCC/ml). This can be seen in Figure 1 where we have both a vertical and horizontal color gradient. Especially for current cell counts between 300,000 and 3 million SCC/ml lower CDI are associated with lower probabilities and with a rising CDI the probability of sustained cell count increases (up to 80%).

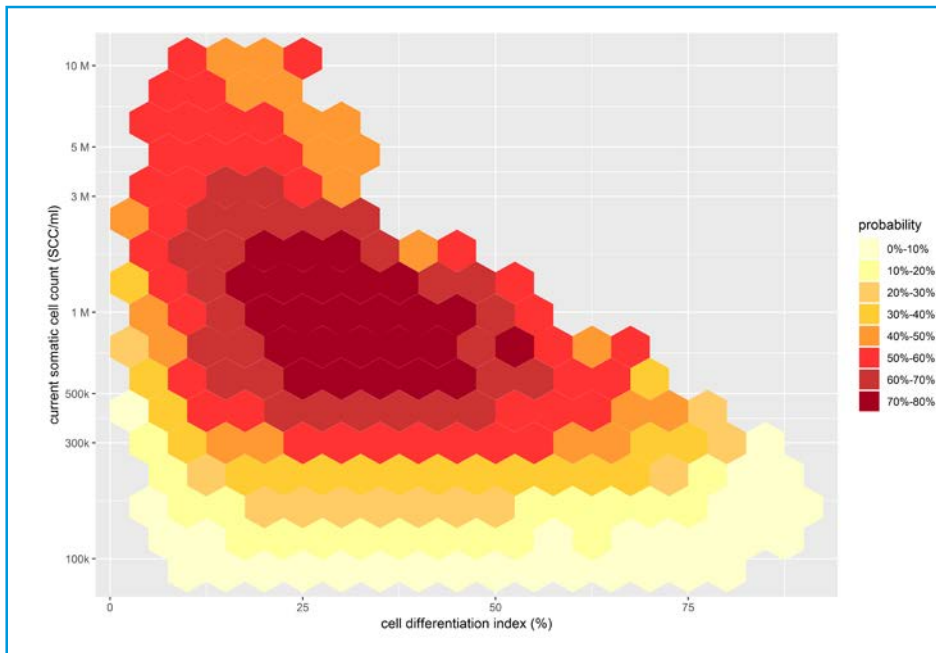


Figure 1. Probability of chronic udder impairment. Cell count above 200,000 cells/ml in the next two months, depending on cell differentiation index (CDI %) and initial cell count above 100,000 cells/ml.

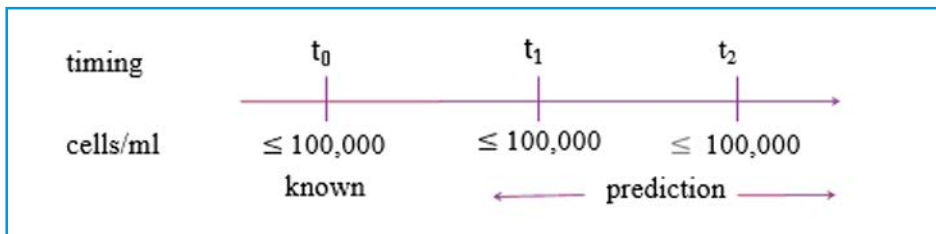


Figure 2. Model for stable udder health: For every animal with a current SCC  $\leq 100,000$  cells/ml, the probability for low cell counts in the next two months is calculated.

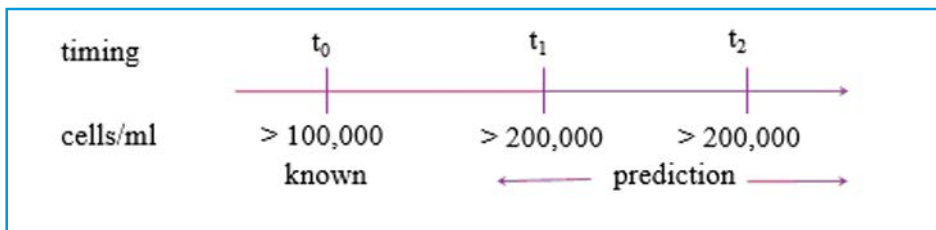


Figure 3. Model for chronic udder impairment: For every animal with a current elevated SCC  $> 100,000$  cells/ml, the probability for high cell counts in the next two months is calculated.

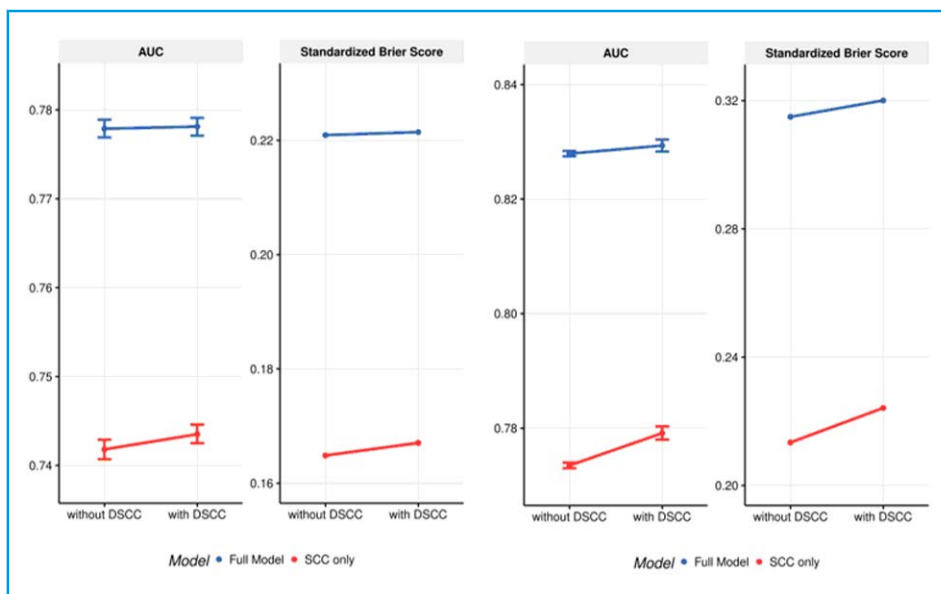


Figure 4. Comparison of model accuracy for different models for (left) stable udder health (Figure 2) and (right) chronic udder impairment (Figure 3) with and without CDI. SCC-only models (red) include current and historic cell counts. Full models (blue) include additional DHI information such as: milk yield, DIM, age, lactose content, fat:protein ratio, proportion of udder healthy cows and new infection rate on the farm.

The predictions of the models accurately reflected the real probability, independent of region, size and breed composition of a farm as tested by multiple validation approaches. The AUC of the chronic udder health model at a SCC threshold of 400,000 cells/ml was 0.868 [95% CI 0.866 – 0.870] with a calibration slope of 0.995 [95% CI 0.983 – 1.006]. For the stable udder health model, the AUC was 0.780 [95% CI 0.779 – 0.781] and the calibration slope was 0.993 [95% CI 0.990 – 0.996].

## Conclusion

As shown in Figure 1, the probability for cell counts > 200,000 cells/ml in the next two months can depend on the level of CDI, especially for animals with current cell counts between 300,000 and 3 million cells/ml. This additional value can be confirmed by values for AUC and standard brier score for the SCC-only model (Figure 4, red). However, when improving the overall model performance by including other available DHI results, the additional value of CDI is minimal (Figure 4, blue).

Recently the full models for stable udder health and for chronic udder impairment were presented and discussed among pilot farms. They were described to be a helpful tool in order to rank animals with different individual risks and to facilitate management decisions, such as the treatment with antibiotics. With these pilot farms a practical evaluation was started in April 2019.

As reference for udder health, an experiment including 2,500 dairy cows was conducted over a period up to 6 months: SCC and cell differentiation were analysed from DHI samples as well as from quarter level samples of the same animals. Additionally, mastitis pathogens were identified from quarter level samples. The data of this experiment will be analysed shortly, allowing to better characterize the additional value of CDI.

## Outlook

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**Damm, M., C. Holm, M. Blaabjerg, M. N. Bro and D. Schwarz, 2017.** Differential Somatic Cell Count - A Novel Method for Routine Mastitis Screening in the Frame of Dairy Herd Improvement Testing Programs. *J. Dairy Sci.* 100(6): 4926-4940.

**Pilla R., M. Malvisi, G. G. Snel, D. Schwarz, S. König, C. P. Czerny and R. Piccinini, 2013.** Differential Cell Count as an Alternative Method to Diagnose Dairy Cow Mastitis. *J. Dairy Sci.* 96(3): 1653-1660.

**Wood, S. N., 2008.** Fast Stable Direct Fitting and Smoothness Selection for Generalized Additive Models. *J. of the Royal Statistical Society: Series B (Statistical Methodology)* 70(3): p 495-518.

## List of references

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