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Clinical predictive significance of biomarker molecules elevation during the transition period in cattle suffering from different pathological states: A review

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Abstract
The transition period (TP), which extends from 3 weeks before 3 weeks post parturition, is a critical period regarding the health, productivity, and profitability of dairy animals, during which most health disorders arise, including lameness, mastitis, rumen acidosis, ketosis, hypocalcemia (HC) (milk fever), left-displaced abomasum, fatty liver, hypophosphatemia and post-parturient hemoglobinuria, subacute ruminal acidosis, RP, and metritis. Biomarkers are biological molecules distributed in blood, body fluids, or tissues that represent physiological or pathophysiological indicators of events, processes, or conditions happening within the animal's body. In the field of veterinary medicine, biomarkers are thought to have enormous valuable potential in the field of clinical diagnosis, therapeutical research, surgery, and obstetrical outcome. This review article aims to explore the significance of biomarkers used to predict pathological conditions and health status of cattle during the TP to facilitate the early clinical diagnosis and prompt treatment of TP-related diseases/or conditions and thus improve animal welfare and health and increase productivity.
Keywords: Biomarkers, Cattle, Clinical diagnosis, Pathology, Transition period.

Introduction

Biomarkers are biological molecules distributed in the body fluids, blood, or tissues that represent physiological or pathophysiological indicators of events, processes, or conditions happening within the animal's body. In the field of veterinary medicine, the detection of biomarkers is a dramatic emerging and developing research field, that possesses enormous potential in the field of clinical diagnosis and prognosis (Tharwat, 2020; Almundarij and Tharwat, 2023; Tharwat, 2023). This topic is relevant not only to the welfare and health of food production and companion animals but also to wide subjects, such as universal food security. It is believed that future progress in the biomarkers field will ultimately help to reduce the workload within the veterinary field, enhance animal welfare results, and improve the financial viability of the livestock industry (Perera, *et al*., 2023).

The transition period (TP), which extends from 3 weeks before 3 weeks post parturition, is a critical period for the animal health and welfare, production and productivity, and profitability of dairy animals. The majority of health disorders arise during this period (Drackley, 1999; Lean, *et al*., 2013; Tharwat, *et al*., 2015a; Tharwat, *et al*., 2015; Tharwat *et al*., 2024). At this phase, the animal undergoes a group of physiological, social, and nutritional changes, and is more susceptible to stress, noninfectious and infectious disorders (Elshafey, 2023). Metabolically, the animal is in a condition of mobilization of nutrient store, principally that of fat and alterable protein. The effective transmission of the animal from the pregnant to the post-parturient state as well as the calved dam demands tremendous physiological adaptations against many stress factors to which the dam and fetus are exposed during this critical period (Ospina, *et al*., 2013). The prosperity or failure of this TP similarly commands the offspring's existence and subsequent recovery of the dam (Lean, 2013). In addition, during TP, many systems including the nervous system are greatly mobilized to supply the optimal circumstances for embryonic growth, the nursing of neonates, and production requirements (Skotnicka, *et al*., 2011). Therefore, the TP remains "the final outlines" for study in the field of dairy animal sciences (Mezzetti, *et al*., 2021).

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TP is also distinguished by severe changes in the endocrine state of the animal, that are more dramatic compared to those that occur during the gestation-lactation cycle. The feed intake is also reduced, at a time when the nutrient needs for the developing fetus and starting lactogenesis are high (Drackley, 1999). The TP is the most stressful period within the production cycle of a dairy animal, due to the decreased feed intake and metabolic and endocrine changes that occur just before or post-parturition. For an optimal and safe transition, a deep understanding of the biochemical variations that occur at the TP is required (Drackley, 1992). Metabolic stress occurs when the physiologic homeostasis is disrupted due to chronic inflammation, oxidative stress, and/or irregular metabolism. Dairies in an early-lactation phase that experience metabolic stress are more vulnerable to health problems, that cause significant loss and decrease in their production (Putman, *et al*., 2018).

During the TP, the animal must adjust to a dramatic, multifaceted growth in nutritive uptake by the udder, which is linked with milk formation, versus the smaller nutrient demand of the developing conceptus during late pregnancy. Thus, this period is associated with an increased incidence of metabolic and production-related diseases, that arise due to the inadequate homeostatic adaptation of the metabolism (Tharwat, *et al*., 2012a; Tharwat, *et al*., 2012b; Thawat and Al-Sobayil, 2015a, 2015b). These terms, combined with the variation in hormonal status, lead to increased mobilization of fat resulting in high levels of nonesterified fatty acids (NEFAs) and a higher level of fatty acid stored in the liver (Grummer, 1995). When the level of liver triglycerides (TG) formation increases the level of TG removal, the stored of cholesterol esters and TG in the liver cells occurs, leading to a critical risk of development of fatty liver syndrome. TG may disappear due to hydrolysis or secretion via the formation of very low-density lipoproteins (VLDLs) (Oikawa, *et al*., 2010). Relative to nonruminants, the ruminant animals have the same level of TG formation in the liver but a very slow level of hepatic VLDL removal (Pullen, *et al*., 1990). A severe economic loss, due to settlement of production and reproduction, may result from the suboptimal transition of the pregnant animal between the late-gestation period and lactation (Drackley, 1999; Overton and Waldron, 2004). A safe transition from late pregnancy to early lactation is vital for ensuring good productivity and reproductive efficiency during the later post-partum period in dairies. Therefore, understanding the eulogies and consequences of the metabolic variations that occur at TP is vital for management of the postpartum animal health (Wankhade, *et al*., 2017).

The most common managemental diseases that occur during TP are lameness, mastitis, rumen acidosis (RA), ketosis, hypocalcemia (HC) (milk fever), left-displaced abomasum, fatty liver, hypophosphatemia and post-parturient hemoglobinuria (PPH), subacute ruminal acidosis, retained placenta (RP), and metritis (Bezerra, *et al*., 2014; Sundrum, 2015). Periparturient diseases can be prognosticated in dairy cows before their onset by using a multimetabolic biomarker model (Hailemariam et al., 2014). Huge direct and indirect economic losses result from these diseases. The direct costs are expenditures on factors such as veterinarians' and managers' labor, medication, and supplies, while the indirect losses include reduced milk production, nonsaleable milk, increased risk of culling, reduced reproductive performance, and reduced animal welfare (Liang, *et al*., 2017).

This review article explores the significance of biomarker elevation during various pathological states that occur during the TP in cattle. It is believed that the large-scale application of these biomarkers will facilitate the early detection and prompt treatment of TP-related diseases and thus improve animal welfare.

Biomarkers that are employed to predict pathological

Worldwide, dairy cows are among the highly intensively farmed animals. High-producing dairy cows have been genetically chosen for their high level of milk yield, which raises the tendency of them to develop special diseases, such as lameness, ketosis, RA, mastitis, HC (milk fever), left-displaced abomasum, fatty liver, hypophosphatemia and PPH, subacute ruminal acidosis, RP, and metritis. The stabilization of biomarkers for the purpose of early verification of these disorders is currently one of the most substantial aspects of the field of dairy animal research (Zachut, *et al*., 2020).

Employing biomarkers in the blood to diagnose lameness

Lameness is probably the most important animal welfare issue that causes premature and involuntary culling within a dairy herd. It is most likely attributed to laminitis a secondary consequence to high grain feeding or RA (Oetzel, 2007) (Fig. 1). Laminitis is a metabolic disorder of the corium that affects the lamellae of the claw, with sole hemorrhage and white-line defects caused by improper nutrition or bacterial diseases, such as metritis or mastitis. It is associated with lameness and is often a herd problem that occurs around parturition, while individual cases of laminitis are often incidental and more accidental in nature (Kloostermann, 2007; Buch, *et al*., 2011).

Three metabolites (propionyl carnitine, carnitine, and lysophosphatidylcholine acyl C14:0) are markedly increased in unsound cows versus healthy ones as early as 4 weeks before parturition, whereas two metabolites (phosphatidylcholine diacyl C42:6 and phosphatidylcholine acyl-alkyl C42:4) may be used to distinguish healthy from diseased ones 1 week before parturition. A plasma biomarker profile comprising of 3 metabolites was established, that could prognosticate which cows would show periparturient problems, up to 4 weeks before the appearance of clinical manifestations, with 87% sensitivity and 85% specificity. Thus,

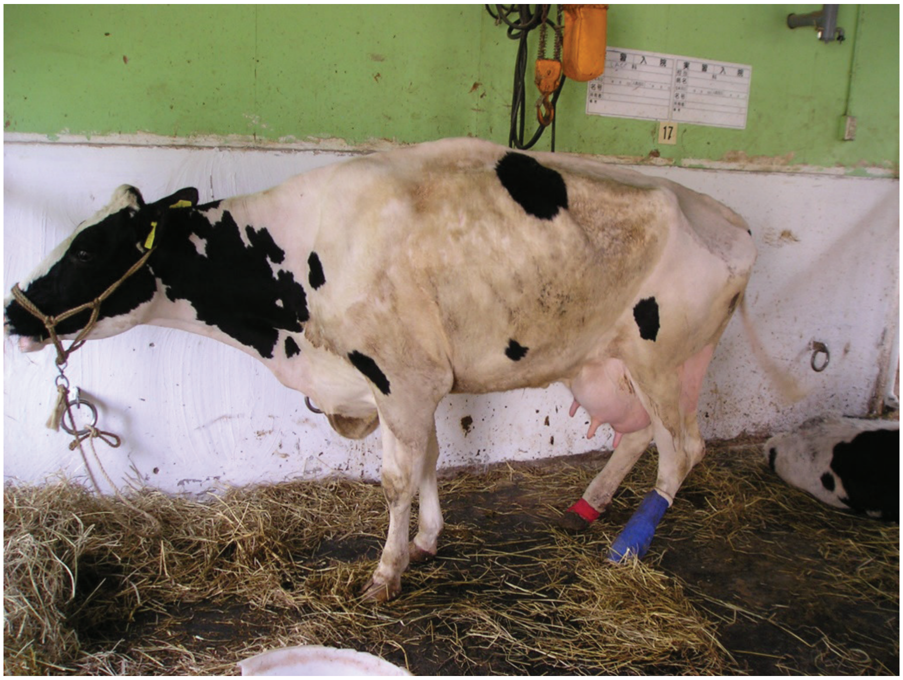

Fig. 1. Lameness in a dairy cow as a cause of premature and involuntary culling.

periparturient problems can be suggested in the dairy cow before their actual appearance, using a multimetabolite marker model (Hailemariam *et al*., 2015).

In cows with subclinical laminitis, the histamine, lipopolysaccharide (LPS), interleukin 6, and tumor necrosis factor-α (TNF-α) are significantly increased ($p < 0.05$), especially histamine, LPS, and TNF-α ($p < 0.01$), compared to healthy cattle. In cows with chronic laminitis, the cyclooxygenase-2 (COX-2), histamine, IL-6, LPS, TNF-α, and inducible nitric oxide synthase ($p < 0.05$) are significantly increased ($p < 0.05$), especially COX-2, histamine, TNF-α ($p < 0.01$), and thromboxane ($p < 0.01$) (Zhang, *et al*., 2020).

Employing biomarkers in the blood to diagnose mastitis

Worldwide, mastitis or inflammation of the mammary tissue is the most common production disease found in dairy herds (Tharwat, 2011; Tharwat and Selim, 2017) (Fig. 2). This disease affects both heifers and multiparous cows and is usually caused by intra mammary infections due to environmental pathogens, especially coliforms. Mastitis is, therefore, one of the most popular and costly production disorders linked to the dairy section. These infections often affect animal welfare and also cause huge financial losses for the stockholders.

Currently, the most sensible methodology for the screening of subclinical as well as clinical mastitis is somatic cell count (SCC), a biomarker of a fundamental immune response which is illustrated by elevating immunoglobulins and SCC, that is transmitted from the blood to the milk at mastitic infection and both have been proposed for predicting the pathogen causing the mastitis. While pathogens identification needs further bacterial culture or molecular methods; namely, polymerase chain reaction.

An elevated oxidation load was found in cows with mastitis versus healthy ones (Laliotis, *et al*., 2020) during the TP. The measured oxidative stress biomarkers in the later study (oxidative stress index, serum antioxidant capacity, and α-tocopherol) exhibited an excellent ability at parturition regarding the prediction and early detection of cows that are at a higher risk of occurring mastitis in the subsequent lactation. In addition, Santos *et al*. (2018) found that dairy cows with greater concentrations of TG and total proteins were at an elevated risk of developing mastitis during the TP while, in contrast, dry period medication reduced such risk. Significant increases in the oxidative stress biomarkers were also found in dairy cattle and buffaloes with mastitis compared to the healthy controls (Sharma, *et al*., 2016).

The abnormalities identified from the chemistry profile of dairy cows with mastitis include HC and increased aspartate aminotransferase (AST), creatine kinase (CK), and lactate dehydrogenase (LDH) activity. The increased AST activity may be due to hepatocellular dysfunction, necrosis of the mammary tissue, and recumbency, HC may be attributable to debilitation and partial anorexia. While, elevated LDH and CK activity may be due to recumbency and damage to the mammary tissue (Tharwat, 2017).

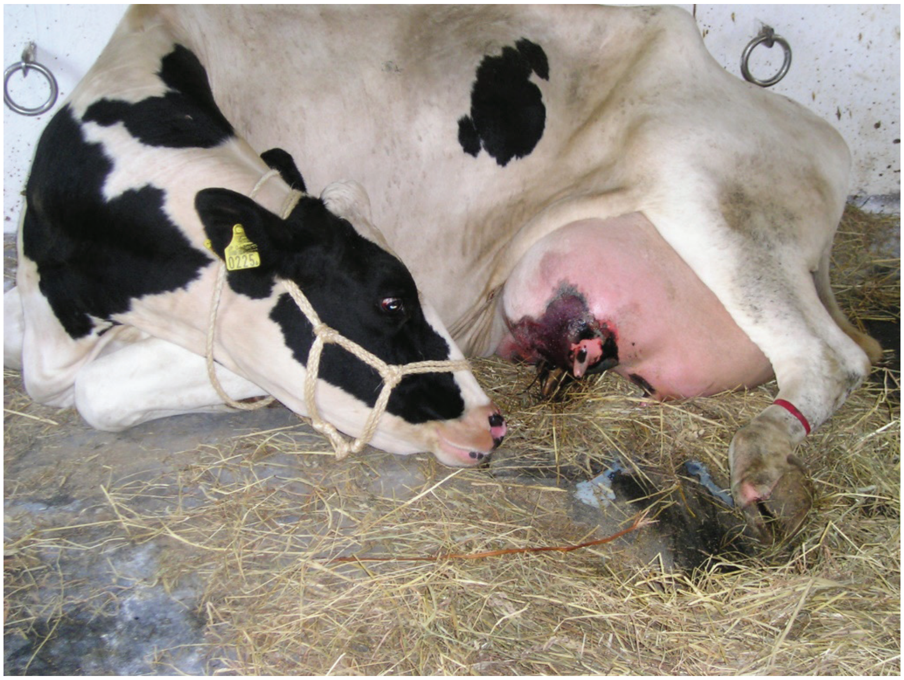

Fig. 2. Holstein cow with *Staph. aureus* peracute mastitis during the transition period with a severe mammary and systemic reaction.

Examining biomarkers in the blood to diagnose rumen acidosis

RA occurs in cattle due to excessive intake of large amounts of concentrated feed, beans, or grain. The increased formation of volatile fatty acids, particularly propionate and butyrate as well as a transient elevation in the ruminal lactate and fluctuation in the osmolality of the rumen juice, may affect the development of the condition and the detachment of the ruminal mucosa (Fig. 3). One of the clearest markers for RA is the remarkable pH value of the ruminal fluid. In real time, a wireless pH probe could be located in the ruminal boluses to provide an immediate ruminal pH measurement, however, pH testing can variate according to the rumen chamber where the probe is located. In addition to ruminal pH, other markers that aid the detection of either subacute rumen acidosis (SARA) or RA can be measured in the urine, blood, milk, or feces. D-lactate has been suggested as a marker for SARA in the blood, as it is wholly formed in the rumen by lactobacilli and bifidobacterium and weakly metabolized by mammals. As a result, D-lactate is raised in the body fluids, such as milk, and can be used as a biomarker for RA diagnosis. Additionally, Danscher *et al*. (2015) stated that RA or SARA did not affect the concentration of milk protein but it reduces the milk fat percentage versus the healthy cows (4.14% and 5.08%, respectively). Therefore, cows with a fat-to-protein ratio below 1:1 in milk may suffer from SARA or even RA.

Examining biomarkers in the blood to diagnose ketosis

Acetonemia or ketosis is a metabolic disorder that is found among high-yielding, lactating animals. It is manifested by inappetence, loss of body weight, drop in milk production, and occasionally nervous symptoms. High-lactating cows, for example, will experience a negative energy balance (NEB) periparturient. This NEB is raised from a fast elevation in the energy demands for milk formation, while the feed intake during early lactation is restricted. Severe NEB around parturition is an important contributor to ketosis (Shen, *et al*., 2021). Two forms exist in cases with clinical ketosis. The first is the digestive form which is characterized by a gradual decrease of body weight over a period of days to weeks (Fig. 4).

In dairy cows, a blood concentration of beta-hydroxy butyric acid (BHBA) >1.4 mmol/l is the common biomarker used for ketosis diagnosis. However, other factors, such as glucose, NEFA, insulin, or glucagon, are also changed during ketosis. In dairy cows, lipid is also mobilized and fatty acid oxidation elevates during the TP, with a following increase in BHBA, thus producing a case of inflammation. As a result, cytokines may be produced stimulating the release of acute phase proteins (APPs) (serum amyloid A (SAA), haptoglobin (Hp), fibrinogen, α1-acid glycoprotein, and ceruloplasmin) primarily from the macrophages. Therefore, APPs and cytokines might be employed as future biomarkers for diagnosing ketosis in dairy cows during the post-parturient phase (El-Deeb and El-Bahr, 2017).

Fig. 3. Rumen acidosis in a Holstein cow where the ulceration and sloughing of ruminal mucosa is apparent.

Fig. 4. Digestive form of ketosis in a cow during the transition period.

Compared to nonketotic cows, dairy cattle with clinical ketosis on the calving date display significantly higher values of mean corpuscular volume, mean corpuscular hemoglobin, NEFA, BHBA, and total bilirubin, but significantly lower values of white blood cells, monocyte, and eosinophil counts, as well as albumin, alanine aminotransferase, LDH, and amylase levels. Therefore, the hematological and serum biochemical parameters can assist in the selection of dairy cows that are liable to ketosis during the TP (Ha, *et al*., 2022).

Ketotic cows show alterations in several variables related to their innate immunity as well as their carbohydrate, amino acid, and lipid metabolism levels weeks before the diagnosis of ketosis. The cytokines (i.e., IL-6 and TNF) and APPs (i.e., SAA, BHBA, and lactate) in the serum were greater in the pre-ketotic cows and ketotic cows compared to the healthy cows. It is, therefore, suggested that the serum levels of pro-inflammatory cytokines (i.e., IL-1, IL-6, and TNF), APPs (i.e., Hp and SAA), and lactate during the dry-off period might be employed as nonspecific screening biomarkers of several periparturient problems among dairy cows during the TP (Zhang, 2016).

Similar to HC, ketosis occurs in cattle when their energy demand exceeds their intake, resulting in a NEB (Zarrin, *et al*., 2013). To prevent ketosis, a sufficient and balanced energetic diet must be provided throughout the pregnancy and postpartum period. When high amounts of rapidly fermentable carbohydrates (i.e., starch) and low fiber content were fed, this may predispose lactating cattle to digestive disturbances mainly RA with the distribution of rumen microflorae populations. Consequently, glucose and lactic acids accumulate, and the ruminal pH decreases (< 4.8) leading to acute RA in which the unaided animal is unable to recover its normal pH levels or SARA where the animal recovers its standard pH levels within a short time. This disorder affects the ruminal and gastrointestinal walls and decreases the blood pH resulting in a state of metabolic acidosis, disturbs nutrient digestion and absorption and predisposes animals to secondary pathologies such as rumenitis, parakeratosis, laminitis, and metabolic acidosis (Kim *et al*., 2021).

Diagnostic biomarkers of milk fever (MK)

Milk fever (MF) or parturient paresis, is a complex metabolic disorder that occurs in dairy cows around calving and is triggered by an imbalance in calcium (Ca) metabolism that occurs immediately before, during, or shortly after calving, although it has also been recorded in dry cows and, increasingly, in the mid-lactation period. Cows with MF are at risk of developing dystocia and metritis, and more vulnerable to other metabolic or infectious diseases, such as abomasum displacement, ketosis, mastitis, uterine prolapse, RP, and a high culling rate during the first 30 days after parturition. Typically, MF consists of three stages: excitation, sternal recumbency, and lateral recumbency (Fig. 5). HC mainly occurs at the initiation of lactation, when the sudden, high need for calcium to be used for milk production by the udder decreases the blood calcium to below 2 mmol/l (subclinical HC) or below 1.4 mmol/l (clinical HC). Clearly, calcium level is the best indicator for diagnosis of HC. However, several other markers can be used, to determine the Ca status in the animal and prevent HC; urine pH is one of these markers (Thilsing-Hansen, *et al*., 2002).

Diagnostic blood biomarkers of left abomasal displacement

Left abomasal displacement (LDA) is a common syndrome in high-producing postpartum dairy cows (Sickinger, *et al*., 2018). The rumen is no longer palpable in the left flank, as the displaced abomasum pushes the rumen to the right and distends under the left rib cage (Fig. 6). A multitude of predisposing factors related to the development of this disorder in cows have been proposed, including breed and genetics, early lactation, puerperal diseases, nutrition and body condition and

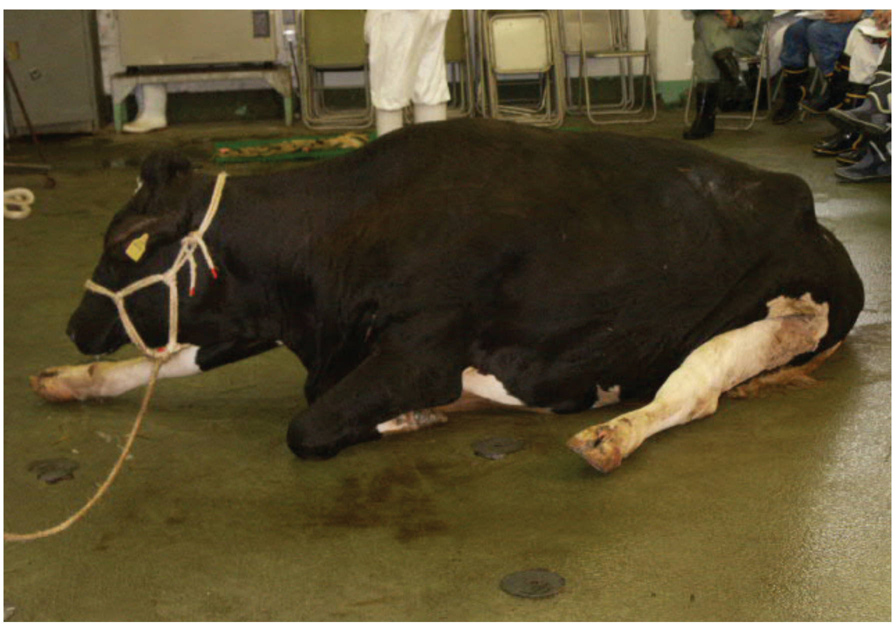

Fig. 5. Second recumbency stage of MF in a cow during the transition period.

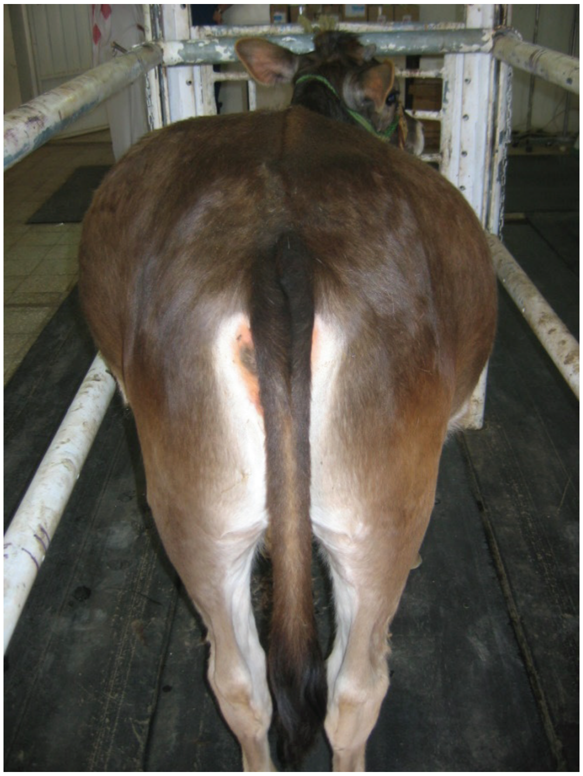

Fig. 6. Left displacement of the abomasum in a cow during the transition period.

electrolyte imbalances (Doll, *et al*., 2009; Zerbin, *et al*., 2015). Abomasal atony, combined with increased gas accumulation and reduced abomasal emptying is regarded as the risk factor for the appearance of abomasal displacement (Doll, *et al*., 2009; Wittek, *et al*., 2005).

In a study of 25 Holstein and crossbreed cows with LDA, aged between 3 and 7 years old, the laboratory findings showed that the packed cell volume %, hemoglobin, total leukocytic count, neutrophils, total protein, AST, glucose, and urea concentrations were significantly elevated versus the controls, whereas a marked decrease in electrolytes (hypochloremia, hyponatremia, HC, and hypokalemia) was found in 88%–92% of the cases with LDA (Mokhber Dezfouli, *et al*., 2013). Common laboratory findings by a retrospective study of 19 beef cattle with abomasal displacement and abomasal volvulus leukocytosis were neutrophilia, hyperglycemia, azotemia, hypochloremia, and hypokalemia. In another, large-scale retrospective study of 1,982 dairy cows with abomasal volvulus and displacement of the abomasum, the laboratory data characteristic of abomasal reflux varied in intensity: 83% had hypokalemia, 67% had an increased base excess, 67% had an elevated rumen chloride, and 50% had hemoconcentration (Braun, *et al*., 2022).

In the cows with abomasal displacement to the right side, the serum SAA, Hp, adenosinedeaminase (ADA), myeleperoxidase, malondialdehyde (MDA), and the activity of CK, AST, gamma-glutamyl transferase (GGT), and creatine kinase-MB (CK-MB) increased significantly, as did the serum MDA, Hp, AST, and ADA concentrations in the cows with LDA (Maden, *et al*., 2012). In a study of 69 cows with displaced abomasum, the concentrations of NEFA, BHBA, AST, glutamate dehydrogenase, and Hp were higher, but insulin and cholesterol were lower compared to the control cows. The glucose and fructosamine concentrations were similar for both groups; however, a tendency toward having lower Revised Quantitative Insulin Sensitivity Check Index values was found in the displaced abomasum cows, indicating reduced insulin sensitivity (Stengärde, *et al*., 2010).

Diagnostic blood biomarkers of fatty liver

Fatty liver is a major metabolic disease in high-producing dairy cows, and its percentage could exceed 50% postpartum (Bobe, *et al*., 2004; Shen, *et al*., 2018). The liver is always enlarged and friable, with a distended gallbladder (Fig. 7). The serum concentrations of lipoprotein-associated phospholipase A2, fibroblast growth factor-21 (FGF-21), hemoglobin- α (Hb-α), Hb-β, and total Hb differ between the fatty liver affected cows and controls. The concentration of serum total Hb and FGF-21 were correlated with the concentration of liver TG and showed potential for suspecting liver TG indicating that FGF-21 and total Hb could be used as biomarkers for detecting fatty liver in dairy cows (Shen, *et al*., 2018). Recently, unique biomarker panels, consisting of fatty acids and amino acids, were defined and validated as an effective tool for detecting fatty liver. These biomarkers were able to distinguish the diseased cows with a higher sensitivity and specificity compared to using the traditional biochemical markers (Zhang, *et al*., 2022).

Diagnostic blood biomarkers of post-parturient hemoglobinuria

Post-parturient hemoglobinuria (PPH) is a condition that arises among early lactating cows and is manifested by hemoglobinuria and acute intravascular hemolysis. The disease occurs all over the world and mainly affects mature, high-producing dairy cows during the first 4–8 weeks of lactation (Stockdale, *et al*., 2005). Clinically this condition is characterized by red urine (Fig. 8) and biochemically by a decline in serum inorganic phosphorus (Pi) to below the normal range. This may be a result of an excessive loss of phosphorus (through the milk, feces, or urine), inadequate dietary phosphorus, a compartmental shift of the phosphorus from the extracellular into the intracellular space, or a combination of one of them (Grünberg, 2014). Hematologically, PPH shares the same characteristics as acute intravascular hemolytic anemia. The packed cell volume falls rapidly to its lowest level in 4–9 days following hemoglobinuria. Serum hypophosphatemia

Fig. 7. Postmortem findings in cows with fatty liver. The liver weighed 18 kg and the gallbladder was distended.

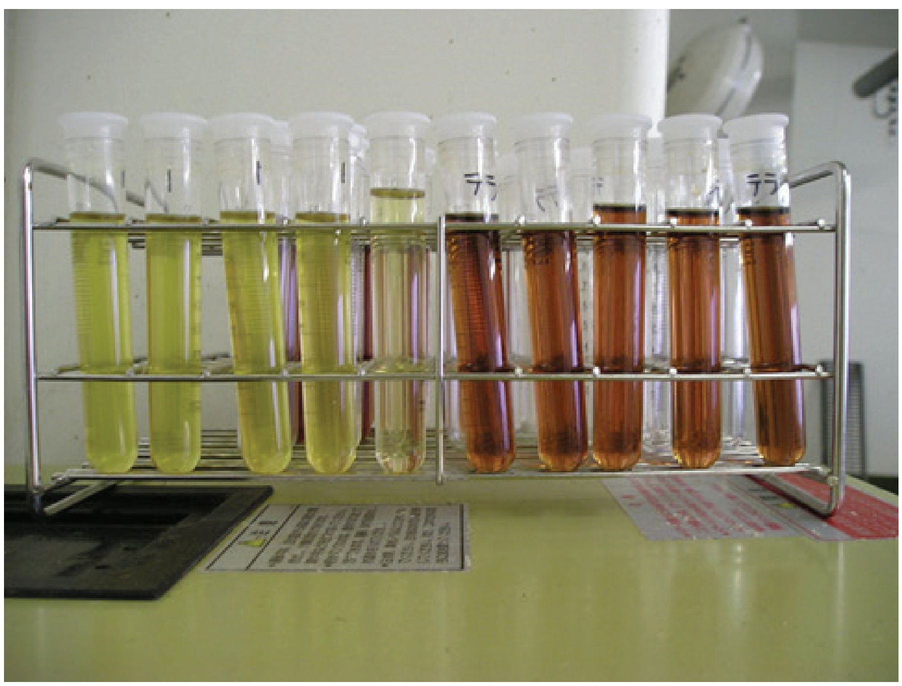

Fig. 8. PPH due to hypophosphatemia in a cow (right) compared to normal urine from a healthy cow (left).

(*Pi* = 0.79 mmoL/l), anemia, and increased liver function analytes (total protein, total bilirubin, and urea concentrations) are also observed in cows with PPH (Abramowicz, *et al*., 2022).

Employing biomarkers in the blood to diagnose subacute rumen acidosis

Dairy cattle are repeatedly fed diets rich in concentrates under intensive systems, especially during their TP.

Cattle that are switched to a such diet may be affected by a short-term leading frequently to the erosion and ulceration of the rumen wall and decreased absorption of nutrients, and therefore lowering productivity. This also affects the ruminal microbial diversity and population and can culminate in the well-known metabolic disorder among high-producing dairy herds during early- and mid-lactation, termed SARA (Minami, *et al*., 2021; Elmhadi, *et al*., 2022). SARA, therefore, is one of the most important gastrointestinal problems associated with intense dairy farming, and the severity of dairy cows' SARA risk may differ between individual animals (Zhang, *et al*., 2022). The disorder of SARA usually decreases feed efficiency, causes chronic health problems, and elevates the environmental effect of milk yield (Oetzel, 2017). In addition, SARA has devastating, long-term health and economic drawbacks for the dairy cow industry such as swinging and depression in feed intake, lowered diet digestion effectiveness, decreased milk fat %, lowered milk production, abscessation of the hepatic parenchyma and lameness (Fig. 9).

Danscher *et al*. (2015) found that SARA was associated with decreased minimum ruminal, fecal, and urinary pH, HC as well as increased PCO_2. The researchers in the latter report concluded that these may be helpful signals of SARA; however, the changes were diurnal. small, and none of these parameters was able to serve as an indicator of SARA independently. In cows that had been subjected experimentally to a sudden, combined, high-grain diet (75% concentrates), with a sudden change of housing system, the SAA, ceruloplasmin, paraoxonase, GGT, and reactive oxygen metabolites increased as positive phase proteins, while the albumin and ferric-reducing antioxidant power decreased as negative phase proteins (Cavallini, *et al*., 2021). It was also reported that the milk urea nitrogen and milk fat % in the late-lactating period in cows that were fed a high-concentrate feeding may be used to identify cows with a high or low risk of SARA (Gao and Oba, 2015).

Employing biomarkers in the blood to diagnose a retained placenta

A retained placenta (RP) where the placenta has not been expelled within 24 hours after parturition is a costly disease that is found among multiparous cows. This disease has been associated with microbial infections, increased lipid mobilization, immunosuppression, and lowered antioxidant status. Different problems can be induced by RP, such as metritis, pyometra, laminitis, and mastitis resulting in considerable financial crises, and especially influencing milk yield and reproductive efficiency (Heinonen and Heinonen, 1989).

Thus, a lower pre-partal body condition score could be used as an early predictor of RP, while decreased α-tocopherol and increased BHBA and NEFA could be used as an early predictor of the disorder (Qu, *et al*., 2014). Lu *et al*. (2018) reported that serum blood urea nitrogen (BUN) at 7 days before parturition may serve as a predictive biomarker of an RP in dairy cows with a predictive critical value of 10.25 mg/dl. When

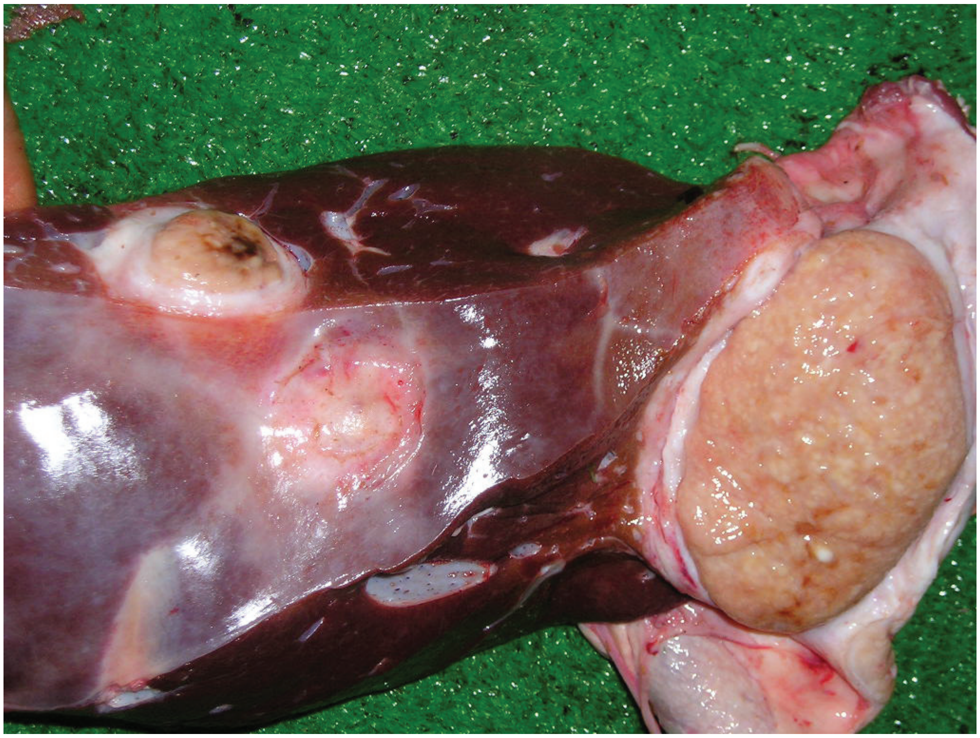

Fig. 9. Postmortem findings of multiple liver abscesses in a dairy cow.

the BUN exceeds the value of 10.25 mg/dl on day 7 before parturition, there is lowered inflammatory cell infiltration in the placentomes and elevated congestion and hyperemia in the small vessels, hence elevating the risk of RP in dairy cows.

Cows with RP had increased concentrations of lactate, SAA, TNF, IL-1, and IL-6 versus the healthy ones. Intriguingly, elevated concentrations of all the previous 5 variables were observed at weeks 8 and 4 before the onset of RP versus the control cows. Animals with RP also display an activated innate immunity 8 weeks before the detection RP, indicating that the 5 variables can be used as biomarkers to suspect cows that might show health issues during the TP (Dervishi, *et al*., 2016). The AST activity, RBC count, and reactive oxygen were significantly elevated in cows with RP versus controls. Opposite, the thiol groups, neutrophils, and serum zinc levels were significantly decreased in the RP cows versus healthy animals (Moretti, *et al*., 2015). Although RP must be defined as a syndrome with multiple etiologies, many of which are linked with parturition or modified metabolic states leading to HC and/or hyperketonemia (Beagley, *et al*., 2010) indicating that neutropenia may act as a co-factor concerning its pathogenesis.

High levels of fatty acids (FAs) and cholesterol were also found in cows with a high risk of RP where there is a 5% increase in the incidence of RP for each 0.1 mmol/l elevation in FAs or cholesterol in the week before parturition. Therefore, levels of FAs cholesterol may be useful for detecting cows with energy imbalance or a metabolic abnormality that might predispose them to RP but should be interpreted in junction with other risk factors, such as dystocia, torsion, or HC (Quiroz, *et al*., 2009). Chebel (2021) reported that the indexes formed by the measurements of the different analyses to suggest the risk of metritis and RP revealed that NEFA, Hp, optical density, and polymorphonuclear leukocyte function may be used as predictors of metritis and RP.

Employing biomarkers in the blood to diagnose metritis

Hailemariam *et al*. (2018) examined the use of serum metabolite panels, at 8 and 4 weeks prepartum, as the risk biomarkers of metritis in transition dairy cattle. These metabolites differentiated cows with metritis versus the healthy ones at 8 weeks prepartum and included ornithine, oxalate, D-mannose, glutamic acid, and pyroglutamic acid. At 4 weeks before parturition, they included pyroglutamic acid, glutamic acid, D-mannose ornithine, and phosphoric acid, suggesting their possible use as risk biomarkers for cows with metritis. In cows and mares with endometritis, immune cells invade the endometrium during inflammation and release bioactive oxidative stress substances to fight secondary or primary or pathogens. These biomolecules may be traced to chemokines, prostaglandins, and cytokines as well as reactive oxygen and nitrogen species (ROS and RNS), which are together known as RONS (Boni and Cecchini Gualandi, 2022).

In conclusion, A lot of research has been carried out for a better understanding of the biology of the dairy cow during the TP. These attempts persist to show higher rates of metabolic diseases that are harmful to the welfare of dairy cattle and also to its productivity with following a significant financial impact on the stockholders. This review is the first that explores the significance of biomarker elevation during certain pathological states within cattle's TP. It identifies the biomarkers that are used to predict pathological conditions during the TP, before discussing in detail those employed to verify the pathological states listed above. It is believed that the large-scale application of these biomarkers during the TP will facilitate the early detection and prompt treatment of TP-related diseases and thus improve dairy cattle welfare.

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Conflict of interest

The authors declare that there is no conflict of interest.

Authors' contributions

MT: Conceptualization, writing manuscript draft, editing, and revising the manuscript. SA and EA: editing and revising the manuscript. All authors revised and approved the manuscript for publication.

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Data availability

All data supporting the findings of this study are available within the manuscript and no additional data sources are required.

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