TUMOUR NECROSIS FACTOR-α
AND INTERLEUKIN-6 CONCENTRATION
IN THE SERUM OF SOWS WITH THE MMA SYNDROME

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Abstract

The investigations were carried out in 10 sows that developed post-partum MMA syndrome (experimental group) and in 10 healthy sows (control) from one closed production cycle farm. The levels of TNFα and IL-6 were measured 12-24 and 48-72 h before, as well as 12-24 and 48-72 h after parturition using the ELISA. The findings revealed significantly increased postpartum levels of TNFα in both groups. Compared to the control group, the levels of TNFα in the experimental group were significantly higher 12-24 h before and 48-72 h after the parturition. The IL-6 levels significantly increased in the experimental group 48-72 h after the parturition. At both postpartum measurement points, the levels of IL-6 were significantly higher in the experimental group than those in controls. The results indicate that TNFα and IL-6 are involved in the pathogenesis of the MMA syndrome and that their determinations are useful for early diagnosis and monitoring of the disease.

Key words: sows, mastitis-metritis-agalactia syndrome, TNFα, IL-6.

The mastitis-metritis-agalactia syndrome (MMA) is the most common post-partum disorder in sows (9). The disease results in substantial economic losses, mainly due to malnutrition of piglets leading to the inhibition of their growth, higher susceptibility to infections, and increased mortality rates. It is widely accepted that the disease is caused by bacterial infection, mainly with Escherichia coli (10, 13). Endotoxin (LPS), released during proteolysis or intensive multiplication of Gram-negative bacteria, is known to have toxic effects on the tissues and to initiate the production of proinflammatory cytokines, which are responsible for the majority of clinical symptoms of diseases caused by these bacteria (1). Numerous experimental studies demonstrated the appearance of MMA symptoms after the administration of E. coli endotoxin to swine (14, 16).

Proinflammatory cytokines are low-molecular-mass proteins (below 50 kDa), which affect many cells of the immune system, thus playing a significant role in acute and chronic inflammatory processes (1). They are produced at the infection site, mainly by activated macrophages, regulate the local inflammatory reaction, and induce the systemic response manifesting itself in leukocytosis, lower blood pressure, fever, anorexia, even shock, and death (6). The production of proinflammatory cytokines is induced shortly after the infection (2, 6). Therefore, determinations of these cytokines may be relevant for early diagnosis of infectious diseases before their clinical symptoms have developed.

The essential proinflammatory cytokines, produced already several hours after the infection, include tumour necrosis factor alpha (TNFα) and interleukin-6 (IL-6) (2). Recently, many authors demonstrated increased levels of these cytokines in sows following experimental infection with Escherichia coli or administration of its endotoxin (16, 18, 20). However, there are no literature data concerning serum levels of proinflammatory cytokines in sows with the MMA syndrome.

The objective of the present study was to determine serum levels of TNFα and IL-6 in sows with the MMA syndrome and to assess the usefulness of such determinations for early diagnosis of the disease.

Material and Methods

Animals. The study was conducted on 60 sows (Polish Large White - PLW, Polish Landrace - PL and PLW x PL) aged 1-3 years. All the animals were from one, closed production cycle farm, in which the herd consisted of 1 200 sows. During pregnancy, the sows received full ratios of feed according to the requirements for nutrients and energy. The water was accessible ad libitum. Approximately 8-10 d before parturition, the sows were transferred to the farrowing house, where they were kept in single pens with grated floors, in which they could only stand or lie.
All the animals underwent clinical examinations during the last 3 d before and first 3 d after the parturition to assess appetite, behaviour, and abnormalities in the individual organs and systems; rectal temperature was taken twice a day (morning and evening). The experimental group included sows developing clinical symptoms of MMA within 48 h after the parturition (10 animals). Ten randomly chosen clinically healthy animals served as controls.

The affected sows were treated intramuscularly with amoxicillin (Betamox L.A., Scan Vet, 1 ml/10 kg b.w.) and oxytocin (Inj. Oxitocini, Biowet Pulawy, Poland, 20-30 iu).

Blood samples, 9 ml, were collected from the anterior vena cava into vacutette tubes (Greiner Labortechnik GmbH, Austria) at 12-24 and 48-72 h before, as well as 12-24 and 48-72 h after the parturition. After centrifugation, the obtained serum was immediately frozen at –76ºC and stored until assayed.

**Bacteriological examination.** The vaginal smears and/or milk samples were collected from the sows with genital discharge and/or mastitis. The material was cultured on the Mueller and Hinton medium with defibrinated sheep blood and incubated at 37ºC for 24 h. Gram-negative bacteria were identified using commercial API 20E tests, whereas staphylococci and streptococci  using PI 20 Staph and API 20 Strep tests (bio-Merieux). The susceptibility of bacteria to antibiotics was determined using the standard disc diffusion method.

**Cytokine determination.** The serum levels of TNFα were determined by the ELISA using the Pig TNFα ELISA kit (Endogen, Inc., USA). The serum levels of IL-6 were determined by the ELISA using the Porcine IL-6 Immunoassay kit (R&D Systems Inc., USA).

The results were statistically analysed defining the arithmetic mean and standard deviation. Differences between groups were analysed using the Student’s t-test. The significance of differences was accepted at P<0.05.

## Results

**Clinical findings.** Based on the clinical examinations, ten sows were classified as affected by MMA syndrome. All the sows showed elevated rectal temperature above 39.8ºC, depression, reduced or lack of appetite, purulent discharge from the vagina, hypogalactia or agalactia. Six sows demonstrated symptoms of mastitis, i.e. oedematous, hardened, reddened, and painful mammary glands.

**Bacteriological findings.** Bacteria were isolated from all vaginal swabs and mastitic milk of the affected sows. The most common bacteria isolated from the vaginal smears were *Escherichia coli* – 7 (70%) cases. Staphylococci were found in 3 (30%) smears. All milk samples from the inflamed mammary gland contained *E. coli*. The microorganisms showed the highest susceptibility to streptomycin, neomycin, and amoxicillin. *E. coli* strains were most resistant to penicillin, cloxacillin, and lincomycin. Staphylococci were mainly resistant to cloxacillin and lincomycin.

**Cytokine determinations.** The results of serum TNFα determinations are shown in Table 1. In both groups, a statistically significant increase in TNFα concentration was observed post-partum compared to the value before the parturition. In the experimental group, the highest level of TNFα (336.34 ± 196.98 pg/mL) was observed 48-72 h after the parturition whereas in the control group during the first 24 h after the parturition (234.19 ± 165.40 pg/mL). At all measurement points, the levels of TNFα were higher in the experimental group compared to the control one. At 12-24 h pre- and 48-72 h postpartum, the differences were statistically significant.

In the group with MMA, the concentration of IL-6 insignificantly decreased 12-24 h before and 12-24 h after the parturition compared to the value at 48-72 h before the parturition (Table 2). At 48-72 h after the parturition, the concentration of IL-6 significantly increased reaching the highest value during the whole examination period (176.10 ± 91.35 pg/mL). In healthy sows, the highest concentration of IL-6 was observed 48-72 h before the parturition (88.27 ± 41.98 pg/mL). On the last pre-partum day, it decreased significantly and remained at that level until the completion of observation. At all measurement points, the IL-6 levels were higher in the experimental group compared to controls. At both postpartum measurement points, the differences were found to be statistically significant.

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>48-72 h before parturition</th>
<th>12-24 h before parturition</th>
<th>12-24 h after parturition</th>
<th>48-72 h after parturition</th>
<th>statistically significant differences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>c</td>
<td>d</td>
<td></td>
</tr>
<tr>
<td><strong>Experimental</strong></td>
<td>87.51 ±49.0</td>
<td>94.65±32.10</td>
<td>316.39±45.80</td>
<td>336.34±196.98</td>
<td>ac***, ad**, bc***, bd**</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>69.60 ±29.75</td>
<td>45.38±25.03</td>
<td>234.19±165.40</td>
<td>186.76±52.46</td>
<td>ac**, ad***, bc**, bd***</td>
</tr>
</tbody>
</table>

ab...cd – statistically significant intergroup differences at individual measurement points; *P<0.05, **P<0.01, ***P<0.001.
The results of the performed study demonstrated the presence of serum TNFα and IL-6 during the last 72 h before and first three days after the parturition both in healthy and in MMA sows. Our findings correspond to the results reported by Zhu (18), who found a constant blood level of TNFα and IL-6 in healthy sows in the last period of pregnancy and once the parturition started. Our post-partum findings revealed that only the concentration of IL-6 maintained at the pre-partum level whereas the concentration of TNFα statistically significantly increased. An increase in TNFα concentration in the post-partum period is likely to be associated with increased capacity of monocytes to produce this cytokine, which was demonstrated by Sordillo et al. (12). The studies in humans show increased serum TNFα and IL-6 levels in women after normal delivery (5). This suggests that an increase in the concentration of cytokine after parturition is probably due to stress during delivery. According to some authors, IL-1β, IL-6, IL-8, IL-10, TNFα, and TGF-β are involved in the regulation of the parturition (3).

Significantly higher levels of TNFα and IL-6 found in MMA sows, compared to those observed in healthy sows, indicate the involvement of those cytokines in the pathogenesis of the disease. Other authors demonstrated increased TNFα and IL-6 levels in sows with induced symptoms of MMA following inoculation of E. coli (18, 20) or administration of E. coli endotoxin (16). Wang et al. (16) showed a rapid increase in plasma TNFα and IL-6 levels in sows three hours after the intramammary administration of endotoxin, which maintained for 12 h. On the other hand, Webel et al. (17) demonstrated the 10-fold increase in TNFα and 200-fold increase in IL-6 in the plasma of swine following intraperitoneal administration of 5 µg/kg of LPS. The concentration of these cytokines did not increase when the dose of LPS was 0.5 µg/kg. Some recent studies showed increased plasma levels of TNFα and IL-6 in sows following intramammary infection with E. coli (18, 19). Twenty-four hours after the infection, the concentration of cytokines was significantly higher in sows with the symptoms of mastitis than in the remaining healthy ones. The examinations of mRNA expression for TNFα and proinflammatory cytokines in the mammary gland of the infected sows suggest local production of cytokines in this gland, which increases during inflammation (19). Some earlier studies showed increased serum TNFα and IL-6 levels in cows with coliform mastitis, which was positively correlated with the severity of mastitis (8).

Among the cytokines involved in diseases caused by Gram-negative bacteria, TNFα is most strongly implicated. It is mostly produced by monocytes/macrophages stimulated by endotoxin and is involved in the induction of other endogenous mediators of inflammation, such as IL-1, IL-6, IL-8, and IFNγ (11). Earlier administration of TNFα antibodies prevented the development of shock after experimentally induced endotoxaemia and decreased the concentration of IL-1 and IL-6 (15). The literature data is confirmed by our findings, according to which a significant increase in TNFα in affected sows was observed earlier than an increase in IL-6. The fact that the concentration of TNFα before the parturition was significantly higher in the experimental group than that in controls suggests that in the affected sows, the uterus and/or mammary gland were infected and MMA started to develop already in the pre-partum period. This corresponds to the results reported by Magnussona et al. (7), who infected sows intramammarily with E. coli at different periods before the parturition and observed that the symptoms of MMA developed post-partum in those animals that were infected during the last 48 h before the parturition. Escherichia coli is regarded the main aetiologic factor of the MMA syndrome, that was confirmed by our study.

According to Fossom et al. (4), the determination of serum IL-6 levels may be a useful index of bacterial infections in sows. They demonstrated that an early phase of Actinobacillus pleuropneumoniae infection caused increased serum IL-6 levels in 80% of affected sows without increased TNFα concentrations. In our study, an increase in serum IL-6 in sows with MMA was

### Table 2
Serum IL-6 concentration (pg/mL)

<table>
<thead>
<tr>
<th>Group</th>
<th>48-72 h before parturition a</th>
<th>12-24 h before parturition b</th>
<th>12-24 h after parturition c</th>
<th>48-72 h after parturition d</th>
<th>statistically significant differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td>93.58 ±72.17</td>
<td>63.27 ±32.67</td>
<td>52.79 ±17.10</td>
<td>176.10** ±91.35</td>
<td>ad′, bd**, cd**</td>
</tr>
<tr>
<td>Control</td>
<td>88.27 ±41.98</td>
<td>48.26 ±17.18</td>
<td>40.11 ±5.25</td>
<td>41.93 ±20.39</td>
<td>ab′, ac**, ad**</td>
</tr>
</tbody>
</table>

Explanations as in Table 1.
slower than that of TNFα and occurred after the parturition, when the clinical symptoms of the disease had already developed. This suggests that in sows with MMA, the level of IL-6 may be a marker of severity of inflammation in the uterus and/or mammary gland rather than a tool for early diagnosis of the disease.

In conclusion, our findings suggest that TNFα and IL-6 are involved in the pathogenesis of the MMA syndrome and their determinations are useful to monitor the course of the disease. The determination of levels of these cytokines, particularly of TNFα, before the parturition, may be relevant for the detection of the infected sows before the development of clinical symptoms, which would enable earlier treatment. In sows with MMA symptoms, the determination of serum TNFα and IL-6 levels may provide information about the severity of the inflammatory process in the reproductive tract and/or mammary gland.

References