Evaluation of the Relationship of Serum Lactate Concentration and Blood pH on Surgical Wound Healing in Cats Submitted to Total Mastectomy as an Animal Model

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Abstract:

Objectives: The study aimed to evaluate the relationship of serum lactate concentration and blood pH on surgical wound healing, using as animal model a sample of 40 female cats (n=40) with diagnosis of mammary gland neoplasia and submitted to unilateral total mastectomy.

Methods: Three different time points were considered: T0 (immediately pre-surgery), T0A (24 hours after-surgery) and T1 (8 days after-surgery). At T0 and T1 the serum lactate and blood pH were analysed. At T0A and T1 the edema and erythema parameters of the surgical wound were evaluated. Three different levels of the wound healing were evaluated at T1 according to the number of stitches removed.

Results: The lactate concentrations were higher in T1 than in T0, contrary to blood pH. Correlations between tissue healing level and the serum lactate, blood pH, edema and erythema were weak (r<0.39 for all).

Conclusions and relevance: It was not possible to establish any relationship between serum lactate concentration and blood pH with the wound healing level, due to the fact that skin pH is not directly influenced by the blood pH. Considering this, the wound healing process is mainly dependent from the injured tissues pH, which can influence directly and indirectly the different steps of the healing process.

Keywords: Cats; Serum lactate concentration; Blood pH; Wound; Healing process

1. INTRODUCTION

Wound healing is a complex physiological process, of great importance in surgery and medicine, involving tissue repair and regeneration and aiming to restore the normal function and structural integrity of tissues after lesional stimulation.[1-4] It can be described as evolving different and very dynamic phases or stages.[5] Generally, four overlapping phases are considered, as follows: 1) the haemostasis and coagulation phase; 2) the inflammatory phase; the 3) proliferative phase; and 4) the remodelling and maturation phase.[6-14] Recognition of these phases, based on macroscopic characteristics, allows the clinician to make an association between the microscopic and biochemical events, thereby directing procedures to the appropriate management of the lesion.[5-8] Several local and systemic (endogenous or exogenous) factors can lead to impaired healing processes.[3,7,13-16] Oxygen (O2) and lactate are essential elements in all stages in the development of the tissues healing.[13,15] In combination with growth factors, lactate stimulates fibroblast proliferation and angiogenesis.[17-19] Furthermore, lactate accumulates in the wound, regardless of the O2 concentration, and will stimulate vascular endothelial growth factor (VEGF) and collagen deposition.[20,21] During the healing process, lactate originates from 1) the fibroblasts, which increase their glycolytic enzymes in hypoxic situations, thereby producing more lactate; 2) cells in division, which produce and release more lactate; and 3) oxidative burst from the leucocytes appearing as a bioproduct.[22,23] In healthy cats, the value of lactate considered normal for daily production corresponds to values < 1.5 mmol/l, reaching 2.0 mmol/l in some particular situations. Also, lactate concentrations are affected by many different variables.[24,25] Lactate and other
products are removed from the tissues by the return blood flow of venulas, promoting therefore a
decrease in the pH values of venous blood, with the establishment of an acidosis in the early stages of
tissue healing process.[26-28] The present study was developed in a sample of 40 female cats (N=40)
with diagnosis of mammary neoplasia and submitted to total unilateral mastectomy with lymph nodes
deflation, and aimed to 1) evaluate the relationship of serum lactate concentration and blood pH on
surgical wound healing in cats submitted to total mastectomy.

2. MATERIAL AND METHODS

This study was conducted on a sample of 40 female subjects (n = 40) of the Felis catus specie,
European Common breed, with diagnosis of mammary gland neoplasia and submitted to total
unilateral mastectomy surgery. The study protocol was approved by the Ethics Committee of Animal
Welfare (CEBEA), Faculty of Veterinary Medicine, University of Lisbon – FMV/ULisboa, and Anjos
of Assis Veterinary Medicine Centre (CMVAA), and simultaneously by the owners of the patients
that signed the consent form authorisation. These animals were regular hospital patients evaluated
with a surgical clinical condition, and at no time were used as experimental animals. The following
study inclusion criteria were considered: all patients were aged between 8 and 14 years, neutered or
non neutered but in anestro phase, the mammary nodules had diagnosed in a maximum 30-day period,
with previous cytological diagnostic of mammary adenocarcinoma, a final anatomopathological
diagnostic of adenocarcinoma tubulopapilary, and did not receive lactate supplementation in the 24 h
before surgery, and did not present any other concurrent disease issues. Two different time points
were considered during the study to harvest a volume of blood sample by venipuncture of the cephalic
vein after a topical application of bupivacaine gel for routine analysis (hemogram and serum
biochemistries), and to measure the serum lactate: T0 (immediately pre-surgery) and T1 (8 days after
surgery). Minimal restraint of the animals was always employed. Immediately after collection, we
proceeded to the analysis of the biological sample, using the i-STAT of Abbott system CG4+ with the
analytical profile measuring the serum L-lactate concentration in units of mmol/l. The lactate oxidase
enzyme, immobilised on the biosensor for lactate selectively, converts lactate to pyruvate and
hydrogen peroxide (H2O2); when released, this is oxidised in a platinum electrode to produce a current
which is proportional to the lactate concentration in the test sample. The reference interval considered
for this parameter was 0.90 to 1.70 mmol/l. Another time point called T0A (first 24 hours after
surgery) was considered to evaluate the edema and erythema levels presented on surgical wound.
Both parameters (edema and erythema) were evaluated from T0A to T1. The wound healing level was
evaluated at T1 considering three different levels according to the fact that all, some and none stitches
were removed from the surgical wound at this time point. This task was always blind and carried out
by the same two researchers. Surgical procedure consisting in a total unilateral mastectomy with
lymph nodes deflation associated was performed by the same surgeon for all patients, in order to
reduce de bias associated with the time consuming or surgery duration, the surgical technique and
the type of surgical defect extent, promoting their similarity in all cats. All patients were submitted to
the same protocols consisting of the following: fluids with NaCl 0.9%, at a maintenance rate of 5 ml/kg/h;
amoxicillin with clavulanic acid (10 mg/kg/Intramuscular [IM]); carprofen (4mg/kg/Subcutaneous);
buprenorphine (0.02 mg/kg/IM); 2% intralesional lidocaine; and transdermal fentanyl. Anaesthesia
was induced with propofol 1% (4–6 mg/kg/Intravenous) and maintained with 2% isoflurane vapourised
in 11 of 100% oxygen/min. Statistical analysis was conducted using R software in version 3.0.1 and
its extension R Commander. Data normality of each tested variable was achieved by using the
Shapiro–Wilks test. The t- test has shown the occurrence of statistically significant changes in serum
lactate, blood pH, edema and erythema values between T0/ T0A and T1. The Pearson coefficient
Correlation (r) was used to assess the association between all the quantitative variables. For analysis
of variance we used one-way ANOVA test in order to check for statistically significant differences in
serum lactate concentrations, lactate variation (lactate ΔT), and wound healing in patients subjected to
total unilateral mastectomy over the time points, followed by the Post-hoc Bonferroni test. The results
were considered statistically significant for p-values <0.05.

3. RESULTS

Characterisation of the sample according to age, body weight, serum lactate concentrations, lactate
variation, blood pH, edema, erythema and wound healing level of the surgical healing are listed in
Table 1. The lactate concentrations was higher in T1 (2.64 ± 0.54 mmol/l) than in T0 (1.62 ± 0.40
mmol/l). Based on one-way ANOVA, it was possible to conclude that there were statistically
significant differences between serum lactate concentrations at T0 and the blood pH, edema, erythema
and wound healing level parameters (p < 0.05 for all) (Table 2). Using the Pearson correlation coefficient (r), it was possible to determine the correlation between the studied parameters at both time points considered (T0/ T0A and T1), which were moderate positive for serum lactate (r = 0.40), edema (r = 0.41) and erythema (r = 0.58), and strong positive for blood pH (r = 0.73) (Figure 1). For all the results were statistically significant (p < 0.01) (Table 2). The one-way ANOVA test was used to study the variance between the lactate T0 concentrations, the lactate variation from T0 to T1 (Lactate ΔT), and the wound healing level and the blood pH, and the edema and erythema parameters between T0/ T0A and T1 (Table 2). For lactate T0 and all the parameters this test showed statistically significant differences: blood pH, edema, erythema and the wound healing level of the tissues healing (p < 0.05 for all). The use of post-hoc Bonferroni test allow us to conclude that from all pairs simultaneously compared significant differences were registered between the following pairs: lactate T0/ blood pH T0 (p<0.01), lactate T0/ blood pH T1 (p<0.01), lactate T0/ blood pH ΔT (p<0.01), lactate T0/ edema T0A (p = 0.03), lactate T0/ edema T1 (p < 0.01), lactate T0/ erythema T1 (p < 0.01), and lactate T0/ wound healing level (p> 0.01)(Table 2). Correlation between serum lactate at T0 and the other parameters was weak for all the parameters, except for edema T1 which was moderate (r = 0.40) (Table 2). For lactate ΔT lactate, the one-way ANOVA test showed statistically significant differences: blood pH, edema, erythema and the wound healing level of the tissues healing (p < 0.05 for all). The post-hoc Bonferroni test allow us to registered significant differences between the following pairs: lactate ΔT / blood pH T0 (p<0.01), lactate ΔT / blood pH T1 (p<0.01), lactate ΔT / blood pH ΔT (p<0.01), lactate ΔT / edema T1 (p < 0.01), lactate ΔT / erythema T1 (p = 0.02), and lactate ΔT / wound healing level (p> 0.01)(Table 2). Correlation between serum lactate ΔT and the other parameters was weak for all the parameters, except for edema T1 which was moderate (r = 0.59) and for erythema T0A which was very strong (r = 0.86)(Table 2). According to results of one-way ANOVA test statistically significant differences were registered between the wound healing level and the blood pH, edema and erythema T0/ T0A and T1 (p < 0.05). The post - hoc Bonferroni test identified which of the pairs were significantly different from each other. Variations were statistically significant between wound healing level and blood pH at T0 (p < 0.01), T1 (p < 0.01) and ΔT (p = 0.04), the edema at T0A and T1 (p < 0.01 for both), and erythema T0A (p< 0.01)(Table 2). Correlation between wound healing level and the other parameters was weak for all the parameters (r < 0.39 for all) (Table 2).
Table 1. Characterization of the sample according to age, body-weight, serum lactate concentration, blood pH and characteristics of the healing process. Also the used healing scale is presented at the end of the table.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N</th>
<th>TP</th>
<th>X ± SD</th>
<th>Reference values</th>
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<td>-</td>
<td>11.13±1.88</td>
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<td>11</td>
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<td>Not neutered</td>
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<td>40</td>
<td>T1</td>
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</tr>
<tr>
<td>pH</td>
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<td>T0</td>
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<td>7.31-7.41</td>
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<td>40</td>
<td>T1</td>
<td>7.24±0.04</td>
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<td>40</td>
<td>T1</td>
<td>0.23±0.43</td>
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</tr>
<tr>
<td>Erythema</td>
<td>40</td>
<td>T0A</td>
<td>1.30±0.70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>T1</td>
<td>0.05±0.62</td>
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<th>Cicatrisation level</th>
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<td></td>
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<td></td>
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<td>&gt;2-5</td>
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<td>Light</td>
<td>Medium</td>
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<td>Some stitches removed</td>
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</table>

T0 (immediately pre-surgery); T0A (first 24 hours post-surgery); T1 (8 days after surgery)

Table 2. The Pearson correlation coefficient (r) between lactate levels at T0, Lactate ΔT and wound healing level and the other parameters. ANOVA One-Way and Post-Hoc Bonferroni tests to evaluate the variance between the lactate levels at T0, Lactate ΔT and cicatrisation with each considered parameter.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Time point</th>
<th>Pearson correlation (r)</th>
<th>t-test p-value</th>
<th>one-way ANOVA</th>
<th>Post-hoc Bonferroni</th>
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<td>-</td>
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<td>pH T0-T1</td>
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<td>0.73†</td>
<td>&lt;0.001</td>
<td>-</td>
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<td>Edema T0A – T1</td>
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<td>&lt;0.001</td>
<td>-</td>
<td>-</td>
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<td>0.58*</td>
<td>&lt;0.001</td>
<td>-</td>
<td>-</td>
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<tr>
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<td>0.19</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Body-weight</td>
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<td>0.21</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>T0</td>
<td></td>
<td>0.17</td>
<td>-</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>pH T0-T1</td>
<td></td>
<td>0.31</td>
<td>-</td>
<td>&lt; 0.05</td>
<td>&lt;0.01</td>
</tr>
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<td>pH T0-T1</td>
<td></td>
<td>0.05</td>
<td>-</td>
<td>&lt; 0.01</td>
<td>&lt;0.01</td>
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<tr>
<td>Edema</td>
<td></td>
<td>0.22</td>
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<td>Edema T0A – T1</td>
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<td>-</td>
<td>&lt; 0.05</td>
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<td>&lt;0.01</td>
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<tr>
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<td>-</td>
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<td>Body-weight</td>
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<tr>
<td>T0</td>
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<td>Edema T0A – T1</td>
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<td>0.59*</td>
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<td>&lt;0.01</td>
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<tr>
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<td>-</td>
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<tr>
<td>T0</td>
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<td>Edema</td>
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<td>&lt;0.01</td>
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<tr>
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</table>

* - moderate (positive) relationship, †-strong (positive) relationship, Bolt – statistically significant results; T0 (immediately pre-surgery); T0A (first 24 hours post-surgery); T1 (8 days after surgery)
Evaluation of the Relationship of Serum Lactate Concentration and Blood pH on Surgical Wound Healing in Cats Submitted to Total Mastectomy as an Animal Model

4. DISCUSSION

A mastectomy is a surgical procedure characterised by the exeresis of a variable amount of mammary gland tissue, and represents the first choice and the main approach for treating mammary neoplasia.[29] This type of cancer is the third most common in cats, occurring in middle-aged to old animals between 8 and 12 years, and about 90% of neoplasms are malignant.[30-32] The mean age of the patients sample (11.1 years old) was within the interval cited by the literature, as well as for the histopathological neoplasm characterisation, considering that all patients of the sample presented a tubular adenocarcinoma which in cats, represents the most frequent type of mammary gland tumors.[33] These cats were subjected to the same surgical procedure performed by the same surgeon, showing therefore similar results regarding the extent of the surgical defect and the surgery duration. In this study, serum lactate was measured in the pre-operative phase (T0) and 8 days after surgery (T1) in patients submitted to total unilateral mastectomy surgery. Considering that the mean period of time necessary for a wound being totally cover by granulated tissue during the healing process is about 7.5 to 10 days in the cat,[34-39] T1 time point was considered as 8 days after surgery, allowing us to investigate a possible correlation between the serum lactate concentrations and blood pH with the wound healing process in each patient. Blood sample analysis was performed using the i-STAT from Abbott with the analytical profile CG4+ system; with its pH reading, this allows the L-lactate concentrations to be adjusted to a neutral pH (7.4). Lactate concentrations are affected by many different variables. In order to avoid potential changes in lactate either during or after sample blood collection, special procedures should be carried out. In the study we used venous blood samples in order to evaluate the acid–base balance, since they are easier to obtain and more accurately reflect the body’s real metabolic state. [25] Considering that cats are very sensitive to stress, which may promote higher lactate concentrations, the venous blood samples used were collected in a calm environment with minimal restraint of the animals, without using a tourniquet or immediately after its application due to the anaerobic environment that is created. All the samples were analysed immediately after collection, because the lactate can increase by 70% within 30 minutes at 25°C as a result of glycolysis.[40] With the descriptive analysis of the lactate evaluated with the i-STAT system using CG4+, it was found that serum lactate concentration rise after the mastectomy procedure. At T0, the patients presented an average which is within the reference interval, contrary to the average lactate value at T1 that was higher than the maximum reference value.[24,41] This rise in sample was registered in all individuals. Comparing the lactate concentrations obtained at T0 with those obtained at T1, a moderate positive correlation ($p = 0.40$) statistically significant ($p < 0.01$) between the difference in lactate concentrations was achieved. This phenomenon may occur due the lactate T1 values might be influenced by the baseline lactate T0 values. Assuming this, only the lactate T0 concentrations and the lactate variation ($\Delta T$) were used in the study. Patients at T0 presented an average lactate value of 1.62 mmol/L, which is within the reference interval; contrary to patients at T1 which showed an average lactate value of 2.64 mmol/L, showing that patients submitted to total unilateral mastectomy with lymph nodes deflation exhibited hyperlactatemia 8 days after surgery. pH is an important acidity or alkalinity indicator of normal blood. All changes in blood pH, in physiological and pathological conditions, occur through variations in three major variables: the CO2, the electrolyte concentrations, and total weak acid concentrations.[26,42] When CO2 elimination is inadequate relative to the rate of tissue production, will going to increase the $H^+$ concentration inducing a decrease in the pH value.[43-52] pH values below 7.35 are associated with acidemia, whereas values higher than 7.45 are indicative of alkalaemia (7.4 is considered neutral).[49] According to the results, the average of the blood pH at T0 (7.32) was within the reference interval, but slightly below at T1 (7.24) and therefore, patients presented slight acidemia at T1. It is known that blood and tissues pH has a strong impact on cellular functions, enzymatic activity, protein expression and conformation, etc. In intact skin, different products such as the CO2 from oxidative respiration, and the lactate from anaerobic respiration, are removed from the tissues by the return flow of venous blood. An indicator of this is the lower venous blood pH compared to the arterial blood pH.[53,54] When a decrease of tissue blood flow occurs, such as in surgical wounds, ischemia will lead to a lactate increase with an inadequate extraction of metabolites promoting a decrease the local pH level,[26,55,56] which will promote angiogenesis of injured tissues.[57] Also the tissue hypoxia serves as a physiological sign to drive angiogenic response, and to raise lactate production and lower pH.[43,58,59] To assess the healing process, we decided to consider two major signs of inflammatory tissues response, the edema and erythema; which decrease in their final score over time. To
characterize the final wound healing level at T1 we only considered the fact of all, some and none stitches were removed. From the sample, one (n=1) patient didn’t remove stitches, and three (n=3) removed some stitches, all the others (n= 36) removed all the stitches. According to the results, correlations between the lactate T0 concentration and lactate ΔT and the blood pH were weak (r < 39 for all). The same was achieve for correlations between the wound healing level and lactate T0 (r = 0.11) and lactate ΔT (r = 0.04); and wound healing level and blood pH T0 (r = 0.38) and blood pH T1 (r = 0.32).

We can conclude that in cats, it is not possible to establish any relationship between lactate concentration and blood pH with the wound healing level presented by the injured tissues. The main reason for this may be associated with the fact that skin pH is not directly influenced by the blood pH, which presents a very complex system that tightly controls the general body acid-base balance.[60-62] Considering this, the tissues healing process is mainly dependent from the injured tissues pH, which can influence directly and indirectly the different steps of the healing process, and various parameters such as oxygen dissociation, angiogenesis, protease activity, and histotoxicity of bacterial end products,[43,53,63-65] than on serum lactate concentration and blood pH.

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