# Paraneoplastic leukocytosis in a dog following liposarcoma resection

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CASE REPORT

Companion or pet animals

# Paraneoplastic leukocytosis in a dog following liposarcoma resection

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**Abstract**

A 10-year-old, female, neutered cocker spaniel presented for surgical debulking of an axillary and cranial thoracic wall liposarcoma. Pre-surgical blood analysis demonstrated anaemia (packed cell volume 17%), leukocytosis (white blood cell count 43.95 × $10^9$/L) and thrombocytopenia (15 × $10^9$/L), with platelet loss secondary to chronic intra-lesional haemorrhage or immune-mediated destruction, and concomitant *Staphylococcus pseudintermedius* urinary tract infection. A blood transfusion and antibiotics were administered before surgery. Within 48 hours after surgery, an extreme leukocytosis (white blood cell count 170 × $10^9$/L), involving a severe left shift neutrophilia (95 × $10^9$/L) was observed; this resolved within 10 days. Serum granulocyte-colony stimulating factor levels were similar to controls. The extreme leukocytosis was suspected to be related to a paraneoplastic leukaemoid reaction combined with an expected postoperative mild leukocytosis. Further investigation into the pathophysiology underlying similar cases is required. One month after surgery, all haematological abnormalities had normalised, and metronomic chemotherapy with chlorambucil commenced.

**KEYWORDS**
dogs, haematology, oncology

## BACKGROUND

Paraneoplastic syndromes encompass a wide range of neoplasia-related structural and functional changes in the body, mediated by various small molecules secreted by the primary tumour or its metastases.[1] Paraneoplastic syndromes can manifest in a variety of ways, including hypercalcaemia, erythrocytosis, hypoglycaemia and leukocytosis.[2] Granulocyte-colony stimulating factor (G-CSF) is a myelopoietic cytokine, which along with others (such as granulocyte-macrophage colony-stimulating factor [GM-CSF], IL-1, IL-3, IL-6 and TNF-$\alpha$) has previously been reported to be significantly upregulated in leukaemoid paraneoplastic syndromes in a number of cancers in people, including sarcomas, carcinomas, mesotheliomas and melanomas.[3] In people, a paraneoplastic leukaemoid reaction (PLR) is uncommon and is defined by a white blood cell count (WBC count) of greater than 50 × $10^9$/L in the presence of a solid tumour that is not infiltrating the bone marrow.[4] This is often, but not always, accompanied by a left shift neutrophilia. PLR is predominantly driven by significant upregulation of the myelopoietic cytokines G-CSF and GM-CSF. The associated extreme leukocytosis can result in neutrophil counts in excess of 100 × $10^9$/L and may mimic primary myeloproliferative disorders (particularly chronic granulocytic leukaemias), severe infection/inflammation or drug-induced reactions.[3,5] The resulting increase in blood viscosity may lead to a hypercoagulable state that is associated with poorer outcomes. As solid tumours are typically the source of myelopoietic cytokine production in PLR, once these tumours have been removed/treated, the leukaemoid reaction typically wanes and the WBC count can return to normal.[3] However, severe leukaemoid reactions can cause significant anaesthetic and surgical challenges, meaning that tumour resection is not straightforward.

In the veterinary literature, PLR is a rarely reported condition, most frequently seen in dogs with carcinomas.[6] PLR mediated by increased circulating concentrations of G-CSF and IL-6 has been reported in two dogs with primary lung adenocarcinomas and in a cat with a mammary carcinoma, in which biopsy samples were positive for GM-CSF following immunohistochemistry.[7,8]

## CASE PRESENTATION

A 17 kg, 10-year-old, female, neutered English cocker spaniel presented with a history of a left axillary subcutaneous mass, first noted 6 months previously, which progressively involved the left cranial thoracic wall and impaired mobility. A biopsy performed by the referring veterinary surgeon was consistent with a liposarcoma. In the days leading up to referral, the dog developed reduced appetite, lethargy, bruising of the skin overlying the mass and pale mucous membranes.

On presentation, the dog was quiet, alert and responsive, with a body condition score of 6/9. The heart rate was 164 beats per minute and was regular, with mildly hyperdynamic, synchronous peripheral pulses. The mucous membranes were pale pink, with a capillary refill time of 2 seconds. The dog was panting, with normal thoracic auscultation and effort. The rectal temperature was 38.9°C. A 13 × 12 cm semi-firm subcutaneous mass with ecchymoses involving the overlying skin was identified (Figure 1a,b). All peripheral lymph nodes were within normal limits. The rest of the clinical examination was unremarkable.

## INVESTIGATIONS

Blood analysis performed 1 week before surgery (Figure 2) included a complete blood cell count, which revealed moderate to severe normocytic, hypochromic regenerative anaemia (packed cell volume [PCV] 17%; reference interval [RI]: 39%–55% and reticulocyte count of $163 \times 10^9$/L; RI: 0–$60 \times 10^9$/L). It also identified leukocytosis (WBC count $43.95 \times 10^9$/L), consisting of moderate neutrophilia ($28.57 \times 10^9$/L; RI: 3.60–$12.00 \times 10^9$/L) with severe left shift (non-segmented neutrophil count of $10.55 \times 10^9$/L; RI: 0–$0.10 \times 10^9$/L), mild monocytosis ($2.20 \times 10^9$/L; RI: 0–$1.50 \times 10^9$/L) and severe thrombocytopenia ($15 \times 10^9$/L; RI: 200–$500 \times 10^9$/L). In a blood smear, there were no platelet clumps, and the findings were consistent with a regenerative anaemia, with moderate to marked red blood cell hypochromasia, suspected to be due to iron-restricted erythropoiesis (true vs. functional iron deficiency). Scant spherocytes and schistocytes were also present and, in the context of a negative in saline agglutination test, were attributed to red cell fragmentation.

Serum biochemistry revealed a total protein count within the lower reference interval (58.0 g/L; RI: 58–73 g/L [0.58 g/dL; RI: 0.58–0.73 g/dL]), mild hypoalbuminaemia (25.3 g/L; RI: 26–35 g/L [0.25 g/dL; RI: 0.26–0.35 g/dL]), mild elevation in alanine transaminase (266 U/L; RI: 21–102 U/L) and moderate elevation in alkaline phosphatase (350 U/L; RI: 20–60 U/L), mild increases in bile acids (19.60 μmol/L; RI: 0–10.50 μmol/L), total bilirubin (7.60 μmol/L; RI: 0–6.80 μmol/L), cholesterol (7.8 mmol/L; RI: 3.80–7.0 mmol/L), triglycerides (1.23 mmol/L; RI: 0.57–1.14 mmol/L) and urea (9.8 mmol/L; RI: 1.70–7.40 mmol/L). Serum electrolyte concentrations were within reference intervals.

Prothrombin time (11 seconds; RI: 11–17 seconds) and activated partial thromboplastin time (84 seconds; RI: 72–102 seconds) (IDEXX Coag DX Analyser) were within reference intervals. The dog was blood typed as a weak DEA 1.1 positive (Alvedia canine blood typing [immunochromatography]).

**LEARNING POINTS/TAKE-HOME MESSAGES**

- Presence of an extreme leukocytosis in a dog diagnosed with a soft tissue mass should raise concern for malignancy and potential paraneoplastic leukaemoid reaction.
- Liposarcomas have a distinctly different biological profile to lipomas. They do not originate from lipomas, can behave like high-grade, aggressive, soft tissue sarcomas and require early surgical resection to avoid extensive local tissue infiltration and potential paraneoplastic leukaemoid reaction.
- Haematology, biochemistry and coagulation assays form an important part of pre-operative patient assessment.
- There is a significant risk of haemorrhage associated in performing surgery in a severely thrombocytopenic patient; however, where such intervention is required to address the underlying cause, excellent communication between clinicians in a multidisciplinary setting—in this case Oncology, Emergency and Critical Care, Anaesthesia and Surgery—is essential to maximise patient outcome in such complex cases.

Urine analysis on a free catch sample was performed with an automated urine sediment and dipstick analyser (IDEXX SediVue DX and VetLab UA Analyser). The urine was cloudy, dark yellow and appropriately concentrated (USG 1.035), with 250 red blood cells/μL, 500 leukocytes/μL and 100 mg/dL protein, consistent with moderate haematuria and inflammation. Sediment examination identified evidence of inflammation with haematuria, pyuria and amorphous crystalline debris. *Staphylococcus pseudintermedius* was cultured from a cystocentesis urine sample; the isolate was sensitive to clavulanic acid-potentiated amoxicillin, and the dog was treated with 250 mg orally twice a day (Synulox 250 mg palatable tablets, Zoetis) of this antibiotic for lower urinary tract infection (UTI).

One week later, on the day before surgery, repeat haematological examination showed mild improvement in the WBC count ($23 \times 10^9$/L), neutrophilia ($16 \times 10^9$/L), thrombocytopenia ($31 \times 10^9$/L) and anaemia (PCV 20%).

Thoracic and abdominal computed tomography (CT) identified a large, poorly defined, infiltrative axillary and cranial thoracic wall mass with fat to soft tissue attenuation and heterogenous contrast enhancement (Figure 3a,b). The pulmonary parenchyma and regional lymph nodes were within normal limits, with no gross metastasis visible. Multiple uroliths were identified within the urinary bladder (largest 1.1 cm diameter), and one within the proximal urethra.

## DIFFERENTIAL DIAGNOSIS

The main differential diagnosis was a liposarcoma with potential chronic intralesional haemorrhage, resulting in hypochromic regenerative anaemia and consumptive thrombocytopenia, plus a concurrent UTI. Haematuria associated

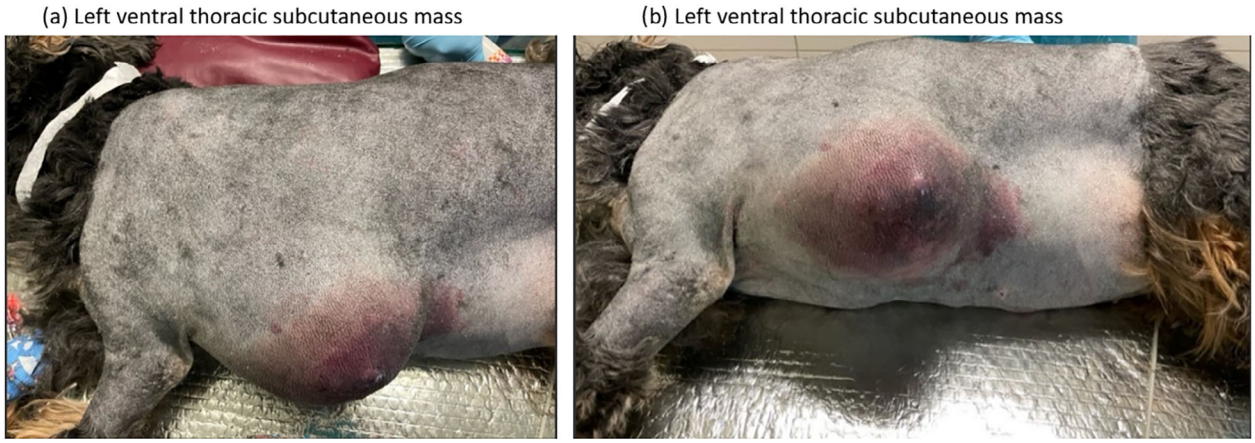


**FIGURE 1** Dorsal plane view of the ventral left thoracic subcutaneous mass with localised ecchymosis (a). Median plane view of the same mass (b).

with UTI was considered to be a contributing factor to these changes. The possibility of an immune-mediated haemolytic anaemia (IMHA) associated with the liposarcoma or UTI was also considered. However, apart from scant spherocytes (which alone has a low sensitivity for detection of IMHA), anaemia of this type can be secondary to oxidative damage or microangiopathic disorders associated with increased red blood cell fragility (localised alteration of blood flow through the liposarcoma); there was no other evidence of haemolysis (ghost cells, haemoglobinaemia or haemoglobinuria).[9] Therefore, the mildly elevated pre-operative bilirubin concentration in the context of regenerative anaemia and predominantly cholestatic elevation in liver parameters was not suspected to be pre-hepatic in origin. Thrombocytopenia was considered likely to be secondary to increased consumption of platelets due to a systemic inflammatory response syndrome (SIRS) and/or intra-tumour haemorrhage.

Differential diagnoses considered for leukocytosis in this case included infection (intra-tumour and urinary tract), inflammation, neutrophil pool redistribution (stress mediated), myeloproliferative neoplasia and paraneoplastic syndrome.

## TREATMENT

The dog was hospitalised and received one unit of DEA 1 negative packed red blood cells (360 mL), uneventfully. The post-transfusion PCV was 34% (14% rise in PCV). The dog's buccal mucosal bleeding time was prolonged (8 minutes; RI: 2–4 minutes),[10] and intermittent bilateral epistaxis was also documented.

Given the nature of the tumour and suspected paraneoplastic response, and after careful consideration involving multidisciplinary discussion surrounding the significant risks of haemorrhage associated with resection, the decision was made to proceed with surgery with preparation of blood products in advance and postoperative care in intensive care unit. The liposarcoma was debulked as much as possible with clear margins not possible given the extent of infiltration into the body wall. The tumour had a dark brown, encapsulated core containing flocculent fluid; the mass was submitted for histopathology and culture. Immediate cytology of the core contents demonstrated many neutrophils and macrophages, with no evidence of infectious agents. A wound soaker catheter for local anaesthetic administration and an active closed suction drain were placed within the surgical site.

Significant spontaneous intra-operative bleeding from multiple sites started to develop during wound closure and was managed with a combination of cautery, compressive swabs and rapid wound closure, alongside administering a unit of fresh frozen plasma. Multimodal analgesia was provided postoperatively, with constant rate infusions of fentanyl and ketamine (starting at 5 μg/kg/h and 5 μg/kg/min, respectively), paracetamol (10 mg/kg intravenously [IV] every 8 hours) and bupivacaine (1 mg/kg every 6 hours) via the wound soaker catheter, with adjustments made depending on serial pain assessments with the Modified Glasgow Composite Pain Scale. Treatment for the previously diagnosed UTI was continued with clavulanic acid-potentiated amoxicillin (20 mg/kg IV every 8 hours; herby UK). The dog developed an accelerated idioventricular rhythm (AIVR) with R on T morphology, which was effectively stabilised on a lidocaine infusion (50 μg/kg/min) within a few minutes and gradually tapered over 48 hours before stopping.

Daily PCV monitoring was performed; on Day 2 postoperatively, the PCV decreased to 18% and the total solids (TS) decreased to 45 g/L. This was suspected to be due to ongoing haemorrhage based on the PCV of the drain fluid (17%) and formation of progressive ecchymoses around the surgical site. The dog was dull, tachycardic (heart rate 160 beats per minute) and hypotensive (systolic blood pressure of 90 mmHg). A second unit of DEA 1 negative packed red blood cells (136 mL) was administered uneventfully. Subsequently, the dog's demeanour and cardiovascular parameters improved with a post-transfusion PCV of 22%; marked neutrophilia with left shift and evidence of toxic change was noted on smear analysis.

Blood was submitted for repeat haematology and biochemistry (Figure 4), demonstrating an increased WBC count ($170 \times 10^9$/L; RI: 6.0-12.0 $\times 10^9$/L), with severe neutrophilia ($95.2 \times 10^9$/L; RI: 3.6–12.0 $\times 10^9$/L), severe left shift (non-segmented neutrophil count 56.10 $\times 10^9$/L; RI: 0–0.10 $\times 10^9$/L), moderate monocytosis (5.10 $\times 10^9$/L; RI: 0–1.50 $\times 10^9$/L), moderate lymphocytosis (10.20 $\times 10^9$/L; RI: 0.70–4.80 $\times 10^9$/L), moderate eosinophilia (3.4 $\times 10^9$/L; RI: 0.0–1.0 $\times 10^9$/L) and ongoing severe thrombocytopenia ($32 \times 10^9$/L; RI: 200–500 $\times 10^9$/L). Given the notable increase in neutrophil count and left shift, ongoing thrombocytopenia, UTI and AIVR, blood cultures were performed to investigate

2) Pre-surgery haematology and biochemistry

| Parameter | Result | Reference Interval and Units |
|---|---|---|
| WBC | 43.95 | 6.00-15.00 x $10^9$/L |
| Neutrophils (segmented) | 28.57 | 3.60-12.00 x $10^9$/L |
| Neutrophils (non-segmented) | 10.55 | 0-0.10 x $10^9$/L |
| Lymphocytes | 1.32 | 0.70-4.80 x $10^9$/L |
| Monocytes | 2.20 | 0-1.50 x $10^9$/L |
| Eosinophils | 1.32 | 0-1 x $10^9$/L |
| Basophils | 0 | 0-0.20 x $10^9$/L |
| PCV | 17 | 35-45 % |
| Hb | 5.10 | 12-18 g/dl |
| MCV | 68.60 | 60-77 fl |
| Platelets | 15 | 200-500 x $10^9$/L |
| Reticulocytes | 163 | 0-60 x $10^9$/L |
| Albumin | 25.30 | 26-35 g/l |
| Globulin | 32.80 | 18-37 g/l |
| ALT | 266 | 21-102 U/l |
| ALP | 350 | 20-60 U/l |
| Bile acids | 19.60 | 0-10.50 µmol/l |
| Bilirubin | 7.60 | 0-6.80 µmol/l |
| Cholesterol | 7.80 | 3.80-7 mmol/l |
| Triglycerides | 1.23 | 0.57-1.14 mmol/l |
| Urea | 9.80 | 1.70-7.40 mmol/l |
| Creatinine | 79 | 22-115 µmol/l |
| Calcium (total) | 2.20 | 2.30-3 mmol/l |
| Phosphate | 1.10 | 0.90-2 mmol/l |
| Sodium | 147 | 139-154 mmol/l |
| Potassium | 3.90 | 3.50-5.60 mmol/l |
| Chloride | 120 | 102-118 mmol/l |
| Glucose | 5.8 | 3-6 mmol/l |

**FIGURE 2** Haematology and biochemistry performed before surgery. Of note is the moderate neutrophilia with marked left shift, moderate to severe regenerative anaemia and marked thrombocytopenia.

whether bacteraemia and/or endocarditis could be driving the profound inflammatory response. Aerobic and anaerobic blood cultures were negative for bacterial growth.

Histopathology of the resected mass confirmed a liposarcoma (Figure 5a,b), with the brown discoloured core containing regions of necrosis and haemorrhage. Aerobic and anaerobic culture of the central core of the mass did not yield any bacterial growth and was deemed sterile.

Analgesia was gradually tapered, and the AIVR had resolved by the time the dog was discharged 1 week after surgery, with a neutrophil count of 77 × $10^9$/L, PCV of 30%, TS of 60 g/L and platelet count of 303 × $10^9$/L.

## OUTCOME AND FOLLOW-UP

Haematology was repeated by the referring veterinary surgeon 10 days following discharge. The PCV was 32%. The postoperative severe leukocytosis had improved (WBC 26 × $10^9$/L), with a mild neutrophilia (13.22 × $10^9$/L) and a marked

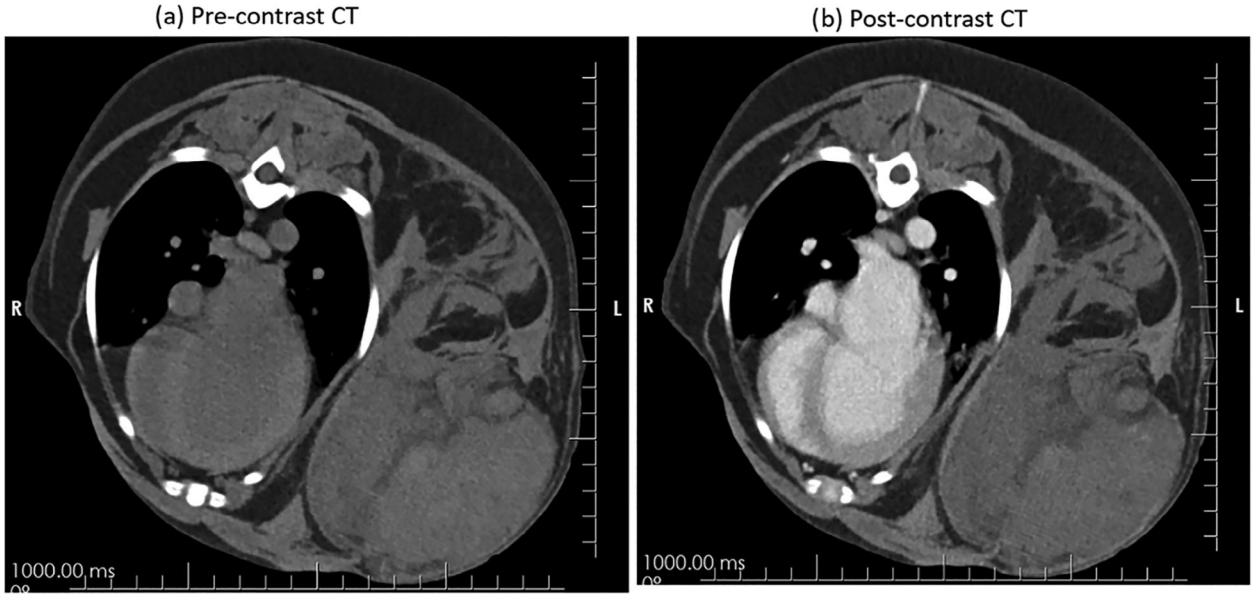


**FIGURE 3** Pre-contrast computed tomography (CT) scan showing a large, ill-defined, heterogeneous, multilobulated mass extending caudally from the third to the 10th intercostal space along the left thoracic wall, with a maximum transverse diameter of 13 cm (a). Post-contrast CT of the mass demonstrates heterogeneous contrast enhancement with poorly defined infiltration into the surrounding tissues (b).

thrombocytosis ($800 \times 10^9$/L), suspected to be mediated by inflammation or due to recovery from the previous thrombocytopenia. The dog was clinically well at a one-month recheck after surgery; haematology performed at this time point showed normalisation of all parameters (WBC $12 \times 10^9$/L), apart from a relapse of mild thrombocytopenia suspected to be secondary to a recurrent UTI.

Given the suspicion of PLR, a G-CSF assay (G-CSF ELISA kit, Enzo Life Sciences, Exeter, UK) was performed 6 months after surgery on pre- and post-surgery serum, which had been stored at −80°C within 24 hours of collection Serum used as a control in this assay was residual from healthy canine serum stored for approximately 6 months under the same conditions and obtained as part of a wellness screening programme at the time of annual vaccination. The serum G-CSF levels in this case were similar to the background level observed in control samples.

As the previously identified uroliths were considered a potential nidus for recurrent UTIs, a cystotomy was performed and two large urinary calculi were removed from the urinary bladder, along with flushing of residual sediment and small stones. One of the large stones was submitted for external composition analysis (Urolith Centre, University of Minnesota Veterinary Diagnostic Laboratory) and the other was crushed in a sterile manner and submitted for bacterial culture. The WBC at the time of cystotomy was within normal limits ($14 \times 10^9$/L). Urolith analysis revealed a mixed composition of predominantly struvite (95%) and ammonium urate (5%). Urolith culture identified the presence of *Staphylococcus aureus*. Concurrent urine culture was negative, with no clinical signs of a lower UTI 1 week post cystotomy; antibiotics were not deemed necessary. Metronomic chemotherapy with chlorambucil was started to delay liposarcoma recurrence and slow metastatic spread; radiation and conventional chemotherapy were considered either too high risk, or of questionable benefit for the dog's disease.

Unfortunately, extensive metastatic disease was identified at a recheck, with repeat thoracic CT scan performed 134 days following liposarcoma resection. Concurrent haematology identified recurrence of anaemia (PCV 18%), leukocytosis ($27.5 \times 10^9$/L) with neutrophilia ($17.5 \times 10^9$/L) and thrombocytopenia ($28 \times 10^9$/L). Palliative management was pursued thereafter.

## DISCUSSION

This case report describes the complex aetiology of a severe postoperative increase in WBC (greater than $50 \times 10^9$/L) in a dog following resection of a liposarcoma, with subsequent normalisation. Factors considered to be contributing to the extreme leukocytosis in this case include: postoperative inflammation, extensive tumour infiltration into surrounding tissues and promotion of an inflammatory microenvironment and manipulation of the tumour during surgical excision (resulting in the secretion of a milieu of inflammatory cytokines). In people, transient postoperative elevation in WBC has been well described in patients undergoing surgery for a variety of reasons; the extent of which is variable and difficult to predict due to its multifactorial nature.[11–13] However, the elevation in WBC in this case is beyond that which can be attributed solely to postoperative inflammation. A case series in people undergoing urothelial carcinoma resection, identified an association between surgical manipulation of the tumour and increased release of G-CSF, in addition to upregulation of G-CSF receptors on the surface of metastases and myeloid progenitor cells.[14]

Liposarcomas are a type of malignant soft tissue sarcoma (STS) originating from lipoblasts and lipocytes most commonly affecting older dogs.[15] Liposarcomas are highly locally invasive; however, the true metastatic potential is unknown.[16] Reported metastatic sites include liver, spleen, lungs and bone.[13] It is important to appreciate that liposarcomas do not arise from malignant transformation of lipomas and they do not share the benign behaviour demonstrated by lipomas; they are a separate entity and behave similarly to highly aggressive

## 4) Post-surgery haematology and biochemistry

| Parameter | Result | Reference Interval and Units |
|---|---|---|
| WBC | 170 | 6.00-15.00 x $10^9$/L |
| Neutrophils (segmented) | 95.20 | 3.60-12.00 x $10^9$/L |
| Neutrophils (non-segmented) | 56.10 | 0-0.10 x $10^9$/L |
| Lymphocytes | 10.20 | 0.70-4.80 x $10^9$/L |
| Monocytes | 5.10 | 0-1.50 x $10^9$/L |
| Eosinophils | 3.40 | 0-1 x $10^9$/L |
| Basophils | 0 | 0-0.20 x $10^9$/L |
| PCV | 0.22 | 0.39-0.55 l/l |
| Hb | 7.00 | 12-18 g/dl |
| MCV | 73.20 | 60-77 fl |
| Platelets | 32 | 200-500 x $10^9$/L |
| Reticulocytes | 221.10 | 0-60 x $10^9$/L |
| Albumin | 20.10 | 26-35 g/l |
| Globulin | 23 | 18-37 g/l |
| ALT | 83 | 21-102 U/l |
| ALP | 321 | 20-60 U/l |
| Bile acids | 9.7 | 0-10.50 µmol/l |
| Bilirubin | 43.80 | 0-6.80 µmol/l |
| Cholesterol | 3.60 | 3.80-7 mmol/l |
| Triglycerides | 0.73 | 0.57-1.14 mmol/l |
| Urea | 5.20 | 1.70-7.40 mmol/l |
| Creatinine | 82 | 22-115 µmol/l |
| Calcium (total) | 2.10 | 2.30-3 mmol/l |
| Phosphate | 1.7 | 0.90-2 mmol/l |
| Sodium | 143 | 139-154 mmol/l |
| Potassium | 3.80 | 3.50-5.60 mmol/l |
| Chloride | 110 | 102-118 mmol/l |
| Glucose | 5.6 | 3-6 mmol/l |

**FIGURE 4** Haematology and biochemistry performed 3 days after liposarcoma resection. The most striking change is a greater than six-fold increase in the segmented neutrophil count after surgery; although an increase in other granulocytes and lymphocyte count is also present.

STSs. Therefore, early surgical resection with the widest possible margins is associated with better outcomes.[17,18]

In this case, the decision was made to proceed with surgical resection, based on both tumour type as well as the suspected paraneoplastic syndrome. However, this decision was not made without consideration of significant risks of haemorrhage associated with surgery in a patient with a marked thrombocytopenia. Caution is advised when considering surgical intervention in cases with severe thrombocytopenia and ideally only when multidisciplinary support is available to manage intra- and postoperative haemorrhage.

Curative intent surgery with appropriate wide and deep margins (which would incorporate forelimb amputation and full thickness thoracic body wall resection) was not considered to be feasible due to the location, size and invasive nature of the tumour, plus the patient's comorbidities. The

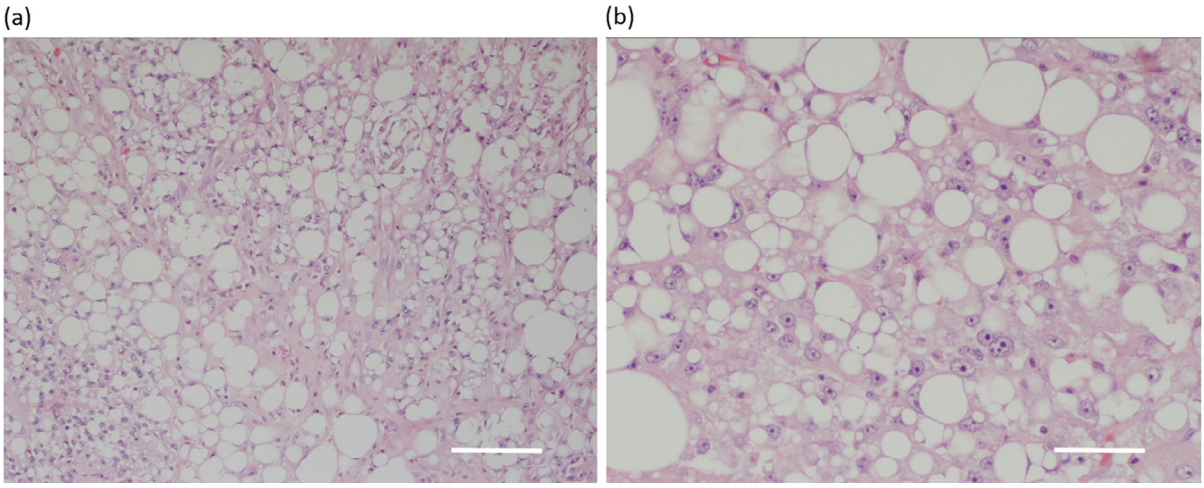


**FIGURE 5** Photomicrographs of histological sections of the liposarcoma, demonstrating sheets of neoplastic adipocytes with formation of clear vacuoles in many cells. Subtle regions of peripheral red-brown colouration most consistent with haemorrhage and necrosis can be seen. Haematoxylin and eosin. Scale bars = 100 μm (a) and 50 μm (b).

agreed surgical aim was planned marginal excision to be followed with adjunctive therapy, with preservation of vital structures within the adjacent brachial plexus to minimise the risk of excessive bleeding or compromising limb function after surgery.

The development of a profound PLR, known as a 'leukaemoid reaction', in association with liposarcomas and other STSs is a rare phenomenon that has been reported in people.[19] A PLR is defined as a persistent leukocytosis with greater than $50 \times 10^9$/L caused by a cytokine secreting tumour, which does not arise from the bone marrow or lymphatic system. Cytokines most commonly implicated in PLR include G-CSF, GM-CSF, IL-1, IL-3, IL-6 and TNF-$\alpha$. G-CSF is a glycoprotein normally produced by vascular endothelial cells, fibroblasts, monocytes and macrophages, which stimulates the maturation of bone marrow progenitor cells into mature neutrophils.[3] In cases of PLR, tumour cells can secrete large quantities of G-CSF and other cytokines, resulting in profound granulocytosis.[20] If confirmed, PLRs are associated with a poor prognosis; a recent retrospective study reported a mortality of 37% (41/111) of dogs with a WBC greater than $50 \times 10^9$/L and underlying neoplastic cause.[21]

It can be challenging to differentiate a PLR from a myeloproliferative neoplasia such as chronic myeloid leukaemia (CML), as both can present with a WBC of higher than $50 \times 10^9$/L and left shift. However, chronic myeloproliferative neoplasia as a cause of a WBC higher than $50 \times 10^9$/L is rare, and cytokine-mediated conditions should be considered as a more likely differential diagnosis, especially without other pertinent clinical findings such as lymphadenomegaly, splenomegaly and hepatomegaly, which could be representative of extramedullary infiltration.[22] It can also be difficult to distinguish PLR from CML based on cytology of bone marrow aspirates alone. Hyperplasia of the affected cell lines can appear similar, regardless of whether it is cytokine driven (PLR) or the result of myeloproliferative neoplasia and, unlike acute granulocytic leukaemia, an increase in myeloblasts is not a feature of CML marrow cytology.[23] Immunophenotyping of bone marrow aspirates/biopsies to assess clonality is often required for a definitive diagnosis of CML.[24] While the authors considered performing a bone marrow biopsy in this dog as part of the initial workup before surgery to exclude myeloproliferative neoplasia, the rarity of this condition, known presence of a large liposarcoma (source of myelopoietic cytokines) in an unwell dog and lack of evidence of extramedullary myeloproliferative disease meant that liposarcoma excision was prioritised. The development of a leukaemoid reaction immediately after surgery, with resolution within 10 days is suggestive of a cytokine-mediated PLR.

Liposarcoma PLR with elevated serum and positive immunohistochemistry staining for G-CSF has been reported in people with pre-surgical elevation of serum G-CSF, which normalises following surgical resection of the liposarcoma.[25] Tamura et al. reported two dogs diagnosed with primary lung adenocarcinoma that had marked leukocytosis and elevated tumour G-CSF and IL-6 gene expression; the leukocytosis in both dogs gradually reduced following tumour resection.[8]

In light of a concurrent postoperative lymphocytosis, it is possible that elevation of other cytokines, such as GM-CSF and IL-6, which were not measured, were contributing to the leukocytosis mediated by receptors, expressed by both granulocytes and lymphocytes.[26] In people undergoing chemotherapy, a significant lymphocytosis after administration of recombinant G-CSF has been documented, and is suspected to be mediated by increased expression of surface cytokine receptor, including G-CSF, by circulating lymphocytes.[27]

Alternatively, G-CSF may be less stable in serum compared to tissue. G-CSF has been shown to have a short serum half-life of 3.5–3.8 hours[28] due to high degradation by proteases, with evidence that storage temperature and multiple freeze–thaw cycles can affect measured concentrations.[29,30] The serum samples submitted for G-CSF analysis were collected 1–3 days after surgery and on two occasions during follow-up visits, when the neutrophilia had resolved, and stored at −80°C before the assay being performed 6 months later. It is possible that due to the timing of the serum sample collection being at the peak and trough neutrophil counts, and the labile nature of G-CSF, significant degradation may have occurred before analysis. Assessment of serum levels of other cytokines that can drive leukocytosis, such as GM-CSF and IL-6, was considered; however, sample quantity, availability and cost of such assays were prohibitive.

While limitations on available resources precluded immunohistochemistry or tissue genetic sequencing of the liposarcoma in this case, this would be an interesting next step to further investigate the cytokine secreting profile given the resolution of the PLR following surgical resection.

## AUTHOR CONTRIBUTIONS

Simone Cutler: primary author, primary resident overseeing ICU care for the case and author that has written most of the manuscript, including an entire first draft, incorporating changes and additional aspects from the others as below. Silvia Caeiro Barreiro: co-author, edited the introduction, the timeline of haematological changes and the section detailing the palliative oncology treatment, for which Silvia was the lead resident. Laura Blackwood: co-author, made significant contributions to both the introduction and the discussion with regards to the paraneoplastic mechanisms and biological behaviour of liposarcomas. Provided some relevant oncology references. Kathryn Pratschke: co-author, performed the surgery to resect the liposarcoma and uroliths and made significant contributions to the surgical management of the liposarcoma in this case, and provided the surgical description and tumour images (provided photographs for Figure 1a,b). Kathryn Pratschke provided surgical insight into the biological behaviour of liposarcomas. Maciej Parys: co-author and performed the serum G-CSF assay and edited the section detailing the methods and interpretation for this. Adrian W. Philbey: co-author, performed the histopathology and provided photomicrographs (Figure 5a,b) and interpretation, as well as comments on how to describe the gross appearance of the liposarcoma. Myrto Spyropoulou: co-author, wrote the blood smear report and also provided advice and edits to the descriptions of the automated haematology and biochemistry, as well as paraneoplastic mechanisms in the introduction. Assisted with formatting of Figures 2 and 4. Daria Starybrat: co-author, senior clinician supervising authors on the case and provided feedback and changes to the intensive care writeup in the case description section, especially with regards to the description of blood transfusions and analgesia management. Craig Breheny: co-author, another senior clinician supervising authors during the intensive care management of the case, provided feedback and modifications to the description and interpretation of haematology findings, and helped to edit the CT image in Figure 3a,b. All of the authors listed above have been actively involved in revising the manuscript, and each has individually given their approval before the final version submitted and have agreed to be held accountable for all aspects of the work.

## CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflicts of interest.

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## ETHICS STATEMENT

Full consent from the owner and the University of Edinburgh Ethics Committee has been obtained for the publication of this case report.

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## MULTIPLE-CHOICE QUESTION

A paraneoplastic leukaemoid reaction (PLR) is defined as a white blood cell count greater than which of the following values?

## POSSIBLE ANSWERS TO MULTIPLE-CHOICE QUESTION

$25 \times 10^9$/L
$50 \times 10^9$/L
$75 \times 10^9$/L
$100 \times 10^9$/L

## CORRECT ANSWER

Correct answer: $50 \times 10^9$/L.

Widely accepted value referenced in,[4] which is also extrapolated from human medical literature.