Peripheral nucleated red blood cells in a cat

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Signalment
Chicca, 2 years old, neutered female, domestic shorthair cat

History
Chicca was presented to the emergency service for severe depression. The owner found the cat in lateral recumbency after being trapped in a mosquito net on a terrace in a sunny summer day. There was no history of recent illness.

Clinical findings
On presentation the cat was markedly depressed and showed severe bradycardia and hyperthermia (rectal temperature= 42°C). She also had severe hypotension (SAP 80 mmHg).

Laboratory findings
Blood samples were sent to the clinical pathology laboratory of the Departement of Veterinary Medical Sciences of the *Alma Mater Studiorum* - University of Bologna, Italy. Venous blood gas analysis showed a severe mixed acidosis with mild hyperlactatemia.
According to the hematological analysis, performed with a Siemens ADVIA 2120 analyzer, Chicca had an increase in the hematocrit, thrombocytopenia and a mild leukocytosis with lymphocytosis and basophilia (Table 1; Fig. 1; Fig. 2). On a May-Grünwald Giemsa stained blood smear no platelet clumps were seen. Numerous nucleated red blood cells (NRBC)(32NRBC/100WBC), mainly metarubricytes, and only few polycromatophils were present (Fig.3; Fig. 4). The basophilia was excluded while the lymphocytosis was confirmed by manual differential count (Table 2).
The serum chemistry profile showed a severe increase of AST activity but only a mild increase in ALT activity, while total bilirubin concentration was WRI. Serum creatinine and urea concentrations were just above the upper reference limit while phosphate was slightly decreased. Hypernatremia, hyperchloremia were also present.
Coagulation tests revealed a mild increase in activated partial thromboplastin time.

Table 1: Results of the hematological analysis.

	Result	Unit	Reference Interval
Hgb	16.7	g/dL	10.0-16.0
Hct	49.0	%	32.0-48.0
RBC	10.44	$x10^6$cells/mm^3	7-11
MCV	46.9	fL	36.0-55.0
MCHC	34.0	g/dL	31.0-36-0
Platelet	72	$x10^3$cells/mm^3	150-500
MPV	16.2	fL	8.0-26.0
WBCB	18550	cells/mm^3	4800-14930
WBCP	18850	cells/mm^3	4800-14930
Neutrophils	21	%	
Lymphocytes	74.2	%	
Monocytes	1.2	%	
Eosinophils	1.1	%	
Basophils	2.4	%	
LUC	0.1	%	
Neutrophils	3890	cells/mm^3	
Lymphocytes	13760	cells/mm^3	900-5600
Monocytes	220	cells/mm^3	0-650
Eosinophils	200	cells/mm^3	1600-10000
Basophils	450	cells/mm^3	
LUC	30	cells/mm^3	
Reticulocytes	68100	cells/mm^3	

Table 2: Results of the differential manual count.

	%	cell/µL
WBC corrected		13759
Neutrophils	27	3872
Lymphocytes	68	9336
Monocytes	5	551
Eosinophils	0	0
Basophils	0	0
37 NRBC/100 WBC		

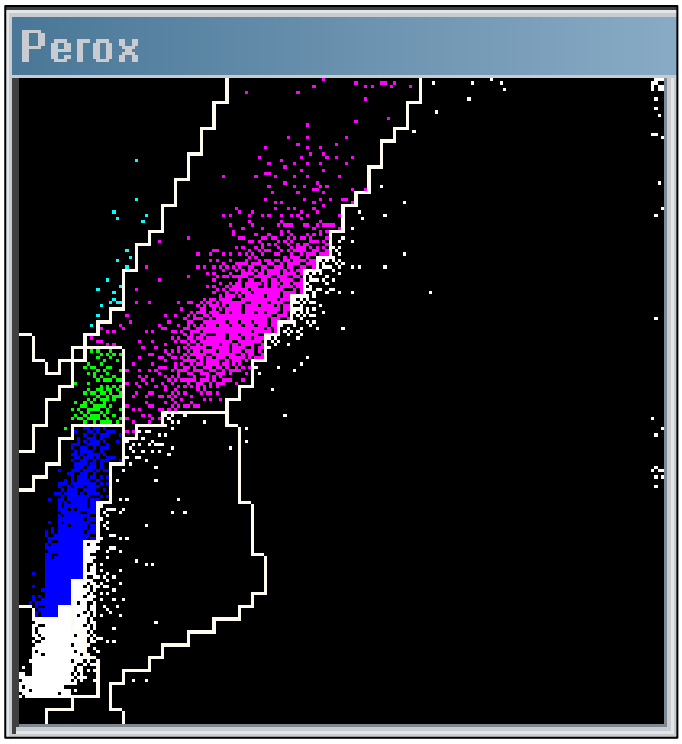

Figure 1: Perox channel cytogram.

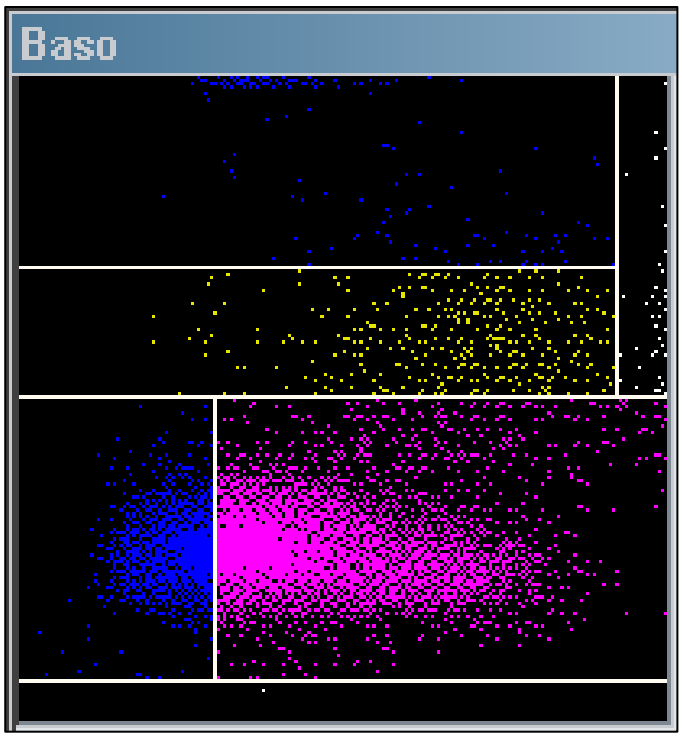

Figure 2: Baso channel cytogram.

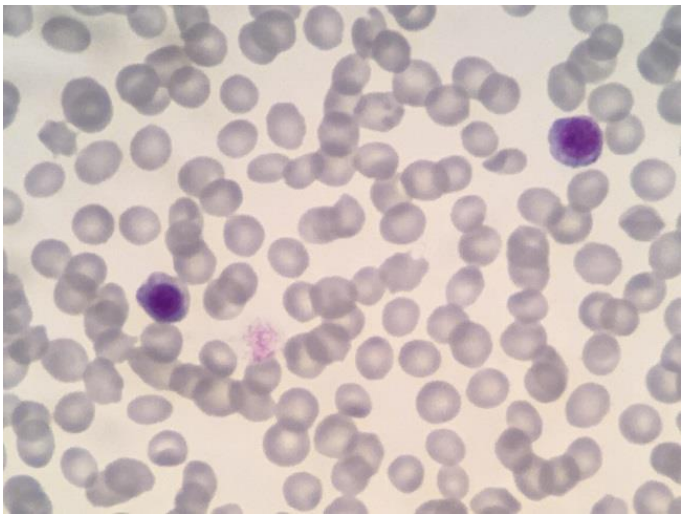

Figure 3: A rubricyte (upper right) and a metarubricyte in the blood smear. May Grünwald-Giemsa (100x).

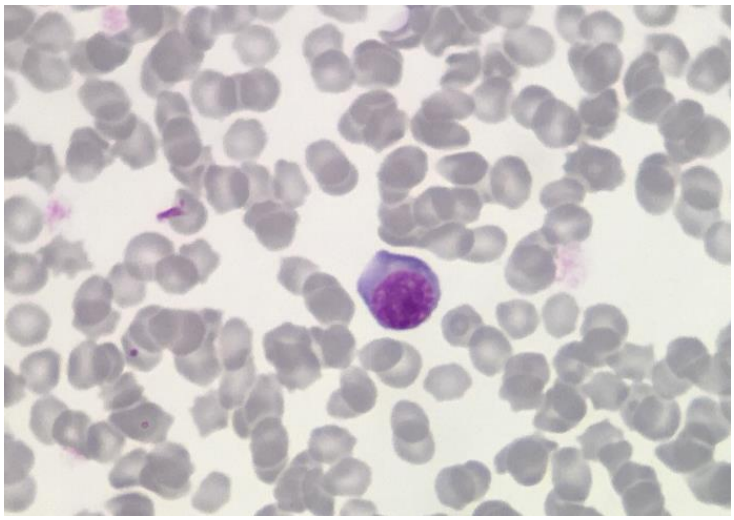

Figure 4: a rubricyte in the blood smear. May Grünwald-Giemsa (100x).

Questions

1. What are your main differential diagnoses?
2. Which is the reason for the lymphocytosis?

Diagnosis
Heat stroke

Discussion

Heat stroke is common in dogs and humans while it is rare in cats; data describing clinicopathological abnormalities in this species lack. When, due to exercise and/or adverse environmental conditions, heat gain exceeds dissipation mechanisms ability, hyperthermia occurs. Direct thermal injury and systemic hypoperfusion, caused by skin vasodilation and fluid loss, are responsible for multiorgan damage and homeostatic systems dysregulation [1].
In the case we reported, several hematological abnormalities were seen. The increase in the hematocrit value may simply be explained by hemoconcentration. Total WBC count, Perox and Baso cytograms, and WBC differential count provided by ADVIA 2120 needed a more accurate interpretation. As expected, blood smear examination only partially confirmed the automated analysis. The presence of numerous NRBC (metarubricytes, rubricytes and prorubricytes) and no basophils made it necessary to perform the manual count, and to correct the WBC count as reported in Table 2.
According to nuclear size and their dense chromatin, metarubricytes are usually included in the "body of the worm" in the Baso cytogram, along with neutrophils. More immature hematopoietic precursors are relatively lysis-resistant cells with poorly lobulated nuclei and are placed in the basophil area [2]. NRBC are excluded [3] or counted as large unstained cell (LUC) or lymphocytes in the WBCP count [2]. Since there was an unremarkable difference between WBCB and WBCP and since we noted a marked reduction of the number of lymphocytes in the manual count compared to WBCP differential count we hypothesized that the NRBC were classified as lymphocytes by the analyzer. The presence of periferal NRBC, unrelated to anemia, is widely described in dogs and humans with heat stroke or heat-related illnesses and in dogs their number is used as a marker for complications and prognosis [4]. Twenty-four hours after admission the number of NRBC notably reduced (Table 3) as already reported in other species [4].
Even after the correction of the WBC count the severe lymphocytosis was still present. Mild lymphocytosis was reported in humans with heat stroke and it was attributed to hemodynamic (increased blood flow in areas usually with low blood flow, such as the skin, and release of local lymphocytes in the circulation) and hormonal (catecholamines) changes [5]. A transient (minutes to hours) increase in lymphocytes and neutrophils counts, induced by catecholamines and hemodynamic changes is possible in dogs and cats but it has never been described in heat stroke. In our clinical case the magnitude of the increase of lymphocytes number was higher than that reported in humans, however in cats an increase of lymphocytes number of up to two folds the upper reference limit might be seen in "physiologic" lymphocytosis [6]. Although this cat did not experience a concomitant increase in neutrophils, the resolution of lymphocytosis 24h after the presentation still support this hypothesis (Table 3).
In humans and dogs nuclear degenerative changes (botryoid nuclei) have been noted in patients with heat stroke but, in this case, on blood smear examination no neutrophils showed such abnormalities.
Thrombocytopenia was confirmed by the absence of platelet clumps on blood smear microscopic evaluation. Disseminated intravascular coagulation is a common complication in heat stroke [7]; coupled with aPTT prolongation and decreased antithrombin activity, the thrombocytopenia of this cat was attributed to platelet consumption caused by heat-induced platelet aggregation and endothelial damage.
Results of serum chemistry profile obtained upon admission and 24 hours after are reported in Table 4. During initial evaluation hypoglycemia (16 mg/dL) was detected via a blood glucose portable meter and the result reported in Table 4 is influenced by glucose administration. Hypoglycemia is often reported in patients with heat stroke and has been attributed both to greater utilization (seizures, respiratory efforts, high body temperature itself) and decreased production (hepatic failure) of glucose. Moreover, sepsis, secondary to

bacterial translocation from damaged gastrointestinal mucosa, may also induce hypoglycemia [8].
Serum creatinine and urea concentrations on presentation were slightly above the reference interval but normalized at the first monitoring after the hospitalization and fluid therapy institution. This apparently reflect a volume-responsive acute kidney injury (AKI). AKI in dogs with heat stroke commonly occurs, it does not only rely on hypoperfusion but even on direct thermal injury, pigment-associated nephropathy and systemic inflammatory response syndrome (SIRS). During heat stroke, AKI has been well documented both with routinely parameters and novel, and more sensitive, biomarkers detecting structural damage even in non-azotemic dogs [9], so, in our case, a structural renal damage could not be completely excluded. Unfortunately, urinalysis was not performed, but it would have been necessary to further characterize the renal damage.
Serum phosphorus concentration was below the reference interval and this could be unexpected during azotemia. In humans with heat stroke hypophosphatemia is often encountered: in a study, 8/10 patients presented with hypophosphatemia and 9/10 had renal function impairment. An increased fractional excretion of phosphorus was detected, therefore, without other signs of tubular disfunction nor hyperparathyroidism, hyperphosphaturia, was considered the most probable cause for hypophosphatemia. In this cat the fractional excretion of phosphorus was not calculated. However, hypophosphatemia might be multifactorial since an intravenous glucose solution was administered, potentially leading to intracellular shift of phosphorus.
ALT and AST activities were markedly above the reference interval. These findings may reflect both muscle and hepatocellular injury. Creatine kinase (CK) activity was measured to confirm muscle involvement; it was extremely high and worsened during the first day of hospitalization. Given the stable severe bradycardia troponin I concentration was also assessed and the result supported cardiac muscle cells damage. The same day mild hyperbilirubinemia developed potentially reflecting the reduction in bilirubin uptake and conjugation by hepatocytes. The cat had no evidence of hemolysis, and biliary obstruction was ruled out. Hepatic, skeletal and cardiac muscle injuries are well described and common sequelae in humans and dogs with heat-related illness [8, 10, 11, 12].
Severe inflammation and SIRS is often reported in people with heat stroke and is believed to play a crucial role in inducing multiorgan dysfunction [13]. In dogs with heat stroke, systemic inflammation has been documented by measuring serum hystones concentration and it has been found that hystones concentration is higher compared to healthy dogs. Moreover, in dogs with heat stroke, hystones concentration was higher in non-survivors [12]. In cats SAA has been proposed as a marker of systemic inflammation and sepsis [14]; in our case SAA was normal upon admission and it was only slightly elevated the day after. It is not known whether in this case inflammation was only mild or potentially the SAA synthesis was reduced due to hepatic damage.

Table 3: result of the hematological analysis upon admission and 24 hours after.

	Admission	24 h	Unit	Reference Interval
Hb	16.7	12.2	g/dL	10.0-16.0
Hct	49.0	34.5	%	32.0-48.0
RBC	10.44	7.5	$x10^6$ cells/mm^3	7-11
MCV	46.9	46	fL	36.0-55.0
MCHC	34.0	35.4	g/dL	31.0-36-0
Platelet	72	8	$x10^3$ cells/mm^3	150-500
MPV	16.2	26.3	fL	8.0-26.0
WBCB	18550	14110	cells/mm^3	4800-14930
WBCP	18850	14480	cells/mm^3	4800-14930
Neutrophils	21	83.2	%	
Lymphocytes	74.2	15.5	%	
Monocytes	1.2	0.5	%	
Eosinophils	1.1	0.6	%	
Basophils	2.4	0.1	%	
LUC	0.1	0.1	%	
Neutrophils	3890	11730	cells/mm^3	
Lymphocytes	13760	2180	cells/mm^3	900-5600
Monocytes	220	70	cells/mm^3	0-650
Eosinophils	200	90	cells/mm^3	1600-10000
Basophils	450	20	cells/mm^3	
LUC	30	10	cells/mm^3	
Reticulocytes	68.1	8.4	$x10^9$ cells/mm^3	
3 NRBC/100 WBC				

Table 4: results of the serum chemistry profile upon admission and 24 hours after.

	Admission	24 h	Unit	Reference Interval
Glucose	426*	96	mg/dL	65-148
Urea	98	37	mg/dL	30-65
Creatinine	1.9	0.73	mg/dL	0.8-1.8
Phosphorus	2.33	2.5	mg/dL	2.5-6.2
AST	554	3209	U/L	9-40
ALT	133	1436	U/L	20-72
GGT	0.1	0.1	U/L	0-4
Alkaline Phosphatase	45	21	U/L	20-140
Total Bilirubin	0.12	0.85	mg/dL	0-0.35
Creatine Kinase	40000	352000	U/L	91-326
Total Calcium	8.8	8.5	mg/dL	6.0-10.5
Total Protein	5.64	5.47	g/dL	6.5-8.8
Albumin	2.65	2.76	g/dL	2.60-4.00
A/G	0.89	1.02		0.52-1.20
Sodium	158	147	mEq/L	145-155
Chloride	129	117	mEq/L	110-123
Troponin I	Not measured	33.7	ng/mL	0-0.20
SAA	1	7	mcg/mL	0-5

*After intravenous 50% glucose solution administration.

Follow up

The cat was immediately cooled and started standard therapies. From the second day of hospitalization Chicca was alert, started to eat and ECG normalized. Serial monitoring showed a progressive improvement of all clinicopathological variables and by day 5 all but ALT, AST and creatine kinase normalized. The same day Chicca was discharged and is actually doing well.

Conclusions

Heat stroke is rare in cats and, to our knowledge, there are no case reported in literature. We provided for the first time a detailed description of clinicopathological findings in a cat presented with severe hyperthermia and central nervous system depression. We found that this cat shared most of the features leading to multiorgan dysfunction recognized in dogs and humans, in particular an increase in muscle, cardiac and kidney injury markers and hemostatic alterations. Moreover, we found that peripheral NRBC, the peculiar hematological abnormality seen in other species, might be an aid in diagnosing heat stroke in cats and that leukocytosis and lymphocytosis may be seen in feline patients with this presentation. Finally, in this cat, SAA only revealed a mild inflammation in contrast to signs and clinicopathological abnormalities of SIRS usually reported in humans and dogs with heat stroke.

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