BIOMARKERS OF NEOPLASTIC TRANSFORMATION IN CANINE SPIROCERCOSIS

by

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Submitted to the Faculty of Veterinary Science, University of Pretoria, in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

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Pretoria, June 2012

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I, Eran Dvir, hereby declare that the work on which this thesis is based, is original and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree at this or any other University, Tertiary Education Institution, or Examining Body.

10 May 2012

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SUMMARY

Spirocerca lupi is a nematode that infects the dog's oesophagus and promotes the formation of an inflammatory fibroblastic nodule that progresses to sarcoma in approximately 25% of cases. Differentiating neoplastic from non-neoplastic cases ante-mortally is challenging and has major therapeutic and prognostic implications. More importantly, spirocercosis-associated oesophageal sarcoma is an excellent and under-utilized spontaneous model of parasite-associated malignancy and the pathogenesis of the neoplastic transformation is poorly understood.

The current study objective was to investigate potential clinical, clinicopathological, radiological and tissue biomarkers for the malignant transformation and an attempt to use these biomarkers to gain a deeper understanding of the pathogenesis of the neoplastic transformation. Our central hypothesis was that the parasite produces excretory product(s) which diverts the immune response from a T helper 1 (Th1) to Th2 cell response, typical of many nematode infections, and further to an immunoregulatory (immunosuppressive), FoxP3+ regulatory T cell-predominated response which then facilitates neoplastic transformation.

The following parameters were studied and compared between cases with non-neoplastic and neoplastic spirocercosis: clinical presentation, haematology, serum albumin and globulin, thoracic radiology, haematoxylin-eosin (H&E) histology, Immunohistochemistry for expression of vascular endothelial growth factor (VEGF)-A, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), MAC387 (myeloid cells), CD3 (T cells), Pax5 (B cells) and FoxP3 (T regulatory cells) and plasma cytokine concentrations including IL-2, IL-4, IL-6, IL-8, IL-10, IL-18, GM-CSF and MCP-1.

Hypertrophic osteopathy showed 100% specificity for neoplastic transformation but relatively poor sensitivity (40%). Female gender, anaemia, leukocytosis, thrombocytosis, spondylitis and bronchial displacement were significantly more common in neoplastic cases, but appeared in non-neoplastic cases as well. The H&E study revealed 2 stages in the non-neoplastic nodules: early inflammation, characterized by fibrocytes and abundant collagen, and a pre-neoplastic stage, characterized by activated fibroblasts and reduced collagen. The neoplastic cases were all sarcomas, primarily osteosarcoma with very aggressive features comparable to other appendicular osteosarcoma in the dog. The inflammation in spirocercosis is characterized by pockets of pus (MAC387+ cells) surrounded by organized lymphoid foci (CD3+ and to a lesser degree Pax5+ cells). There was no evidence of a local accumulation of FoxP3+ cells, unlike many previous studies which have reported an increase in Foxp3+ T cells in both malignancies and parasite infections. Interleukin-8 plasma concentration was higher in the neoplastic group compared to the non-neoplastic and the control groups. Interleukin-18 concentration was higher in the non-neoplastic group followed by the control group and finally the neoplastic group.

As with most similar studies, no ideal biomarker with high sensitivity and specificity was identified. However, if examined together, a panel of the biomarkers that were identified more commonly in the neoplastic cases should substantially increase the index of suspicion for neoplastic transformation in a diagnosed spirocercosis case. The inflammatory response showed features of increased myeloid (innate) response and lymphocytic response with pro-inflammatory cytokines. This was not our initial hypothesis and the question remains whether the response is secondary to the worm infection, or to a symbiotic bacterium that is carried by the worm. The role of such a reaction in neoplastic transformation remains to be elucidated.

ACKNOWLEDGEMENTS

This study was funded by Petplan Charitable Trust, the European College of Veterinary Internal Medicine – Companion Animal (ECVIM-CA) Clinical Research Fund, Duncan Campbell Memorial Fund of the South African Veterinary Foundation (SAVF) and several research funds within the Faculty of Veterinary Science in the University of Pretoria, South Africa (including the Department of Companion Animal Clinical Studies, the section of Pathology within the Department of Paraclinical studies and the Faculty research fund).

I would like to express my gratitude to the following people:

- Srs. Carla van der Merwe, Marizelle DeClerq and Liani Kitshoff of the Onderstepoort Veterinary Academic Hospital (OVAH) for their help in the administration and organisation of the patients and procedures required.
- Carien Muller, Cheryl Pretorius and the other staff in the Clinical Pathology laboratory in the OVAH for their help in the samples preparation and storage.
- Marie Smit in the Immunohistochemistry laboratory in the Faculty of Veterinary Science in the University of Pretoria for her role in the immunohistochemistry staining of the growth factors.
- Jeanie Finlayson, Dr Julio Benavides and the Histopathology laboratory at Moredun Research Institute, and Neil McIntyre at the Royal (Dick) School of Veterinary Studies, Edinburgh, UK for their assistance with the immunohistochemical staining and analysis of the inflammatory cells.
- My colleagues in the section of Small Animal Medicine for their support and hard work on the clinic floor while I was engaged in this project.

- To my residents and my colleagues in the *Spirocerca lupi* research group: Drs. Varaidzo Mukorera, Paolo Pazzi, Chantal Rosa, Liesel L van der Merwe, J Christie and Prof. Robert M Kirberger for their contribution to the clinical management of the cases. Together we made one of the most productive and vibrant research groups in the Faculty.
- My co-authors on the five publications that came out of this PhD: Profs. Robert M Kirberger, Mark C Williams and Johan P Schoeman and Drs. Varaidzo Mukorera, Liesel L van der Merwe, Sarah J Clift, Tom N. McNeilly, Richard J. Mellanby and Mads Kjelgaard-Hansen.
- Prof. Piet Stadler, the former head of department for his support at the beginning of the project.
- My dear friend and my colleague, Prof. Robert M Kirberger, for the continuous support, endless patience, good advices and example of how to combine clinical work with meaningful research.
- My research supervisors, colleagues and friends, Prof. Johan P Schoeman and Dr. Richard J Mellanby for their advice, support and enthusiasm for the project. They have turned obstacles into opportunities to make this project successful.
- Last but not least, to my family, my mother Aliza, my wife Leah and my beautiful two children Yarden and Omer. Their endless support as they went along with their daily life made this project most enjoyable.

ABBREVIATIONS

S. lupi – Spirocerca lupi
µg – microgram
µl – microlitre
BSA - bovine serum albumin
BVSc – Bachelor of Veterinary Science
C – Celsius
C – Collagen
CD - Cluster of differentiation
CRP- C-reactive protein
dl – decilitre
DL – detection limit
DN - degenerate neutrophils
DNA - deoxyribonucleic acid
DVM – Doctor of Veterinary Medicine
Dipl. ECVIM-CA - Diplomate of the European College of Veterinary Internal Medicine – Companion Animals
ED – Eran Dvir
EDTA - Ethylenediaminetetraacetic acid
F – fibroblast
FGF - fibroblast growth factor
Fig. – figure
FoxP3 - Forkhead box P3
GM-CSF - granulocyte-macrophages colony-stimulated factor
H& E / HE - Haematoxylin-eosin
HO - Hypertrophic osteopathy
Hons - Honours
Ht - Haematocrit
IHC – immunohistochemistry
IL - Interleukin
kg – kilogram
L1-5 – Larvae life cycle stage number
LP – lymphocytic-plasmacytic
LSAB - labelled steptavidin-biotin
MAC 387 - Macrophage marker
MCP - monocyte chemotactic protein
Martius, Scarlet and Blue – MSB
MCV - mean corpuscular volume
MCW – Mark C Williams
NF-κB - nuclear factor kappa-light-chain-enhancer of activated B cells
NGS – normal goat serum
Mi – mitoses

ml – millilitre
mm – milimeter
MMedVet – Master in Veterinary Medicine
MN – multinucleated
MVD- Microvessel density
O – osteoblasts
Osm – osteoid matrix
OVAH – Onderstepoort Veterinary Academic Hospital
p – probability
Pax5 - Paired box protein 5
PBS - phosphate buffer
PBST80 - phosphate-buffered saline (PBS) containing 0.5% Tween 80
PDGF - platelet-derived growth factor
pH - power of hydrogen
pg – picogram
RK – Robert Kirberger
RT – room temperature
S. lupi – Spirocerca lupi
SC – subcutaneous
SJC – Sarah J Clift
T – temperature
T1-12 – Thoracic vertebrae number
TGF - Transforming growth factor
Th1 - T helper type
Tregs - T regulatory cells
VEGF - vascular endothelial growth factor
UK – United Kingdom
vs – versus
WBC - White blood cell count
µg – microgram
µl – microlitre
χ^2 - Chi-square

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