The Veterinary Journal 201 (2014) 57–63

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Evaluation of prognostic indicators using validated canine insulinoma tissue microarrays

Floryne O. Buishand [a,*], Judith Visser [a], Marja Kik [b], Andrea Gröne [b], Rebekah I. Keesler [b], Inge H. Briaire-de Bruijn [c], Jolle Kirpensteijn [a]

[a] Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 108, 3584 CM Utrecht, The Netherlands
[b] Department of Pathobiology, Division Pathology, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, 3584 CL Utrecht, The Netherlands
[c] Department of Pathology, Leiden University Medical Centre, Albinusdreef 2, 2300 RC Leiden, The Netherlands

ARTICLE INFO

Article history:
Accepted 1 May 2014

Keywords:
Canine
Insulinoma
Pancreas
Prognostic indicators
Tissue microarray

ABSTRACT

Tissue microarray (TMA) technology allows analysis of multiple tumour samples simultaneously on a single slide. The aim of the present study was to develop and assess a TMA containing 32 primary canine insulinomas and 13 insulinoma metastases. The results of histopathological and immunohistochemical analyses of triplicate core biopsies were compared with those of individual tissue sections using weighted κ statistics. Inter-observer agreement of TMA immunohistochemistry scores were assessed for chromogranin A (CgA), insulin, growth hormone (GH), growth hormone receptor (GHR) and Ki67 index, as well as the prognostic utility of clinicopathological, histopathological and immunohistochemical criteria.

There was substantial agreement of scores for histopathological parameters (κ = 0.64–0.70) and a substantial to near-perfect agreement for homogenous immunohistochemical parameters (κ = 0.69–1.00). Except for GH, which demonstrated heterogeneous staining, there was good to excellent inter-observer agreement for all other immunohistochemical staining scores (intra-class correlation coefficients: 0.70–1.00). On univariate analysis, the presence of nuclear atypia was significantly predictive of disease-free intervals (DFIs) for canine insulinoma, while tumour size, TNM stage, necrosis and Ki67 index were significant in terms of prognosis, with respect to both DFI and survival time. On multivariate analysis, tumour size and Ki67 index retained predictive power for survival time, as did tumour size for DFI. This study confirms the applicability of TMA technology for evaluation of canine insulinoma.

Introduction

Tissue microarrays (TMA) consist of paraffin blocks containing multiple tissue biopsy cores taken from archival samples, which are placed in defined array coordinates (Kononen et al., 1998). Although the TMA technique has been widely used for high-throughput immunohistochemical evaluation of human tumour specimens (Braunschweig et al., 2005; West and van de Rijn, 2006; Alkushi, 2009; Karlsson et al., 2009), there are relatively few reports on their use for evaluation of veterinary tumour samples (Hammer et al., 2007; Keller et al., 2007; Wohlsein et al., 2012).

One major advantage of the TMA technique is that it allows simultaneous evaluation of a relatively large number of tumour samples, stained under identical immunohistochemical conditions (Hassan et al., 2008). Furthermore, using a TMA instead of multiple slides is more cost effective and efficient in terms of reagents, tissue samples and time (Milanes-Yearsley et al., 2002). One disadvantage of the technique is that the small biopsy cores used to construct the TMA might not necessarily be representative of the original tumour sample, particularly when there is substantial intra-tumour heterogeneity (Camp et al., 2008). Additionally, tissue cores potentially can be lost during processing (Eguíluz et al., 2006).

The present study describes construction of TMAs for canine insulinoma (INS), a tumour type that causes clinical signs associated with hypoglycaemia. Although they represent the most common malignant pancreatic endocrine tumour in dogs (Steiner and Bruyette, 1996; Buishand et al., 2013), INS are relatively uncommon canine tumours overall. Development of a TMA could facilitate research to identify histopathological features that might play a role in outcome and survival.

Previously, we have characterised canine INS biopsies using haematoxylin and eosin (H&E) and immunohistochemical staining (Buishand et al., 2012). The aim of the present study was to evaluate use of INS TMAs, compared to individual canine INS sections. Furthermore, reproducibility of immunostaining of the TMAs was determined, comparing scoring results determined by different vet-

* Corresponding author. Tel.: +31 30 2539727.
E-mail address: F.O.Buishand@uu.nl (F.O. Buishand).

http://dx.doi.org/10.1016/j.tvjl.2014.05.004
1090-0233/

erinary pathologists. The prognostic utility of a range of clinico-pathological, histopathological and immunohistochemical criteria was also assessed.

Materials and methods

Study population and samples

Thirty-two primary canine INS biopsies and tissue from 13 accompanying metastatic lesions (10 from lymph node and three from liver) were obtained from the archive of the Department of Pathobiology of the Faculty of Veterinary Medicine, Utrecht University, for construction of the TMAs (Table 1). Of these samples, 29/32 primary canine INS and 8/13 metastases had already been included in previous immunohistochemical studies (Buishand et al., 2010, 2012, 2013). Primary INS tumours had been removed by partial pancreatectomy, using either the suture-fracture technique ($n = 16$) or a vessel-sealing device (LigaSure, Covidien; $n = 15$). One dog (Case 32) was treated medically and did not undergo surgery. After routine processing and paraffin embedding, sections were cut at 5 μm thickness and stained with H&E.

Construction of canine insulinoma tissue microarrays

Two TMA blocks were constructed, one containing biopsy cores from primary INS and the other containing biopsy cores from INS metastases. Both blocks also included biopsy cores from normal canine control tissues, namely pancreas, pituitary, adrenal gland, kidney, heart, jejunum, colon, liver, spleen, lymph node, muscle and lung. The TMAs were constructed by obtaining representative blocks containing INS biopsies and examining corresponding H&E stained slides under light microscopy for foci of high neoplastic cellularity. Multiple representative cores with a diameter of 1.5 mm were taken from the donor blocks (mostly in triplicate; Table 1) and transferred to an acceptor block for processing with an automated tissue array device (TMA Master, 3DHISTECH). The TMA blocks produced were pressed upside

Table 1
Clinico-pathological characteristics of insulinoma biopsies used in the study.

Dog	Breed	Sex	Specimen type	Tumour diameter (cm)	TNM stage[a]	Outcome	DFI (days)	ST (days)	Previous IHC	Cores on TMA (*n*)
Primary tumours										
1	Irish setter	Mx	T	4.0	II	DOD	31	405	In, Ki67	3
2*	Rough collie	F	T	4.0	IV	DOD	122	166	In, Ki67	3
3	Beagle	Fx	T	1.5	I	DOD	161	286	In, Ki67	3
4	German pointer	M	T	2.7	II	DOD	260	777	In, Ki67	3
5*	Labrador retriever	M	T	4.0	III	DOD	462	464	In, Ki67, GH, GHR	3
6*	Belgian shepherd dog	M	T	1.5	III	DOD	510	623	In, Ki67, GH, GHR	3
7	Boxer	Fx	T	3.5[b]	II	DOD	472	561	In, Ki67	3
8	Bearded collie	Fx	T	1.5	III	AWD	546	1045	In, Ki67, GH, GHR	3
9	Jack Russell terrier	Fx	T	3.5	II	DOUC	1042	1042	In, Ki67	3
10	West Highland white terrier	F	T	1.2	I	AW	819	819	In, Ki67	3
11*	Crossbred	Fx	T	0.8	III	AW	1565	1565	In, Ki67, GH, GHR	3
12*	Bearded collie	Mx	T	2.5	IV	DOD	438	647	In, Ki67	3
13*	German shepherd dog	F	T	5.0	IV	DOD	0	0	In, Ki67	3
14	Crossbred	M	T	1.0	I	AW	1154	1154	In, Ki67	3
15	Jack Russell terrier	Fx	T	1.6	I	DOD	213	221	Ki67	3
16	Boxer	Mx	T	2.1	II	DOOD	804	804	Ki67	3
17	Boxer	Fx	T	2.5	III	DOD	509	645	In, Ki67, GH, GHR	3
18*	Kooiker dog	F	T	1.5	IV	DOD	63	442	In, Ki67	3
19	West Highland white terrier	M	T	1.5	I	DOD	343	344	In, Ki67	3
20*	Anatolian shepherd dog	M	T	9.0[c]	IV	DOD	7	69	In, Ki67, GH, GHR	4
21	Crossbred	F	T	1.0	I	AW	1132	1132	NP	3
22	West Highland white terrier	Fx	T	1.3	I	DOOD	385	385	Ki67	3
23	German pointer	Fx	T	3.0	II	AW	512	512	Ki67	3
24*	Labrador retriever	Mx	T	0.3[c]	III	DOD	0	3	GH, GHR	3
25*	Crossbred	Fx	T	3.5	IV	DOD	0	0	GH, GHR	3
26	Crossbred	Mx	T	1.0	I	DOD	1190	1213	Ki67	3
27	Yorkshire terrier	Mx	T	1.0	I	AW	1747	1747	Ki67	3
28	Dachshund	Fx	T	1.3	I	DOD	705	845	Ki67	3
29	Jack Russell terrier	Fx	T	2.0	II	DOD	252	495	Ki67	3
30	German pointer	Mx	T	4.0	III	DOD	200	285	NP	3
31*	German pointer	M	T	3.0	III	DOD	0	249	NP	3
32*	Rough collie	Mx	T	5.0[c]	IV	DOD	0	0	In, GH, GHR	4
Metastases										
2	Rough collie	F	N	3.0	–	–	–	–	NP	2
5	Labrador retriever	M	N	4.0[d]	–	–	–	–	In, GH, GHR	3
6	Belgian shepherd dog	M	N	5.0[d]	–	–	–	–	NP	3
11	Crossbred	Fx	N	2.0	–	–	–	–	In, GH, GHR	3
12	Bearded collie	Mx	M	2.0	–	–	–	–	NP	3
13	German shepherd dog	F	M	5.0	–	–	–	–	In	5
13	German shepherd dog	F	N	2.0	–	–	–	–	NP	3
18	Kooiker dog	F	N	1.0	–	–	–	–	In	3
20	Anatolian shepherd dog	M	N	2.0	–	–	–	–	In	2
24	Labrador retriever	Mx	N	3.0	–	–	–	–	GH, GHR	3
25	Crossbred	Fx	N	5.0[d]	–	–	–	–	GH, GHR	3
31	German pointer	M	N	3.0	–	–	–	–	NP	3
32	Rough collie	Mx	M	5.0	–	–	–	–	In, GH, GHR	3

M, male; F, Female; Mx, castrated male; Fx, female neutered; IHC, immunohistochemistry; DFI, disease-free interval; ST, survival time; T, primary tumour; N, lymph node metastasis; M, distant (liver) metastasis; DOD, died of disease; DOOD, died of other disease; DOUC, died of unknown cause; AW, alive and well; AWD, alive with disease; In, insulin; GH, growth hormone; GHR, growth hormone receptor; TMA, tissue microarray; NP, not performed.

* Indicates cases where a metastases biopsy (N or M) is matched to a primary tumour from the same animal.

[a] Staging was performed according to Buishand et al. (2010).

[b] Two primary insulinomas present.

[c] Multiple insulinoma nodules present, diffusely spread through the left pancreatic lobe.

[d] Multiple lymph node metastases present.