LABOKLIN aktuell

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BRAF mutation and BRAF comp. test – an update

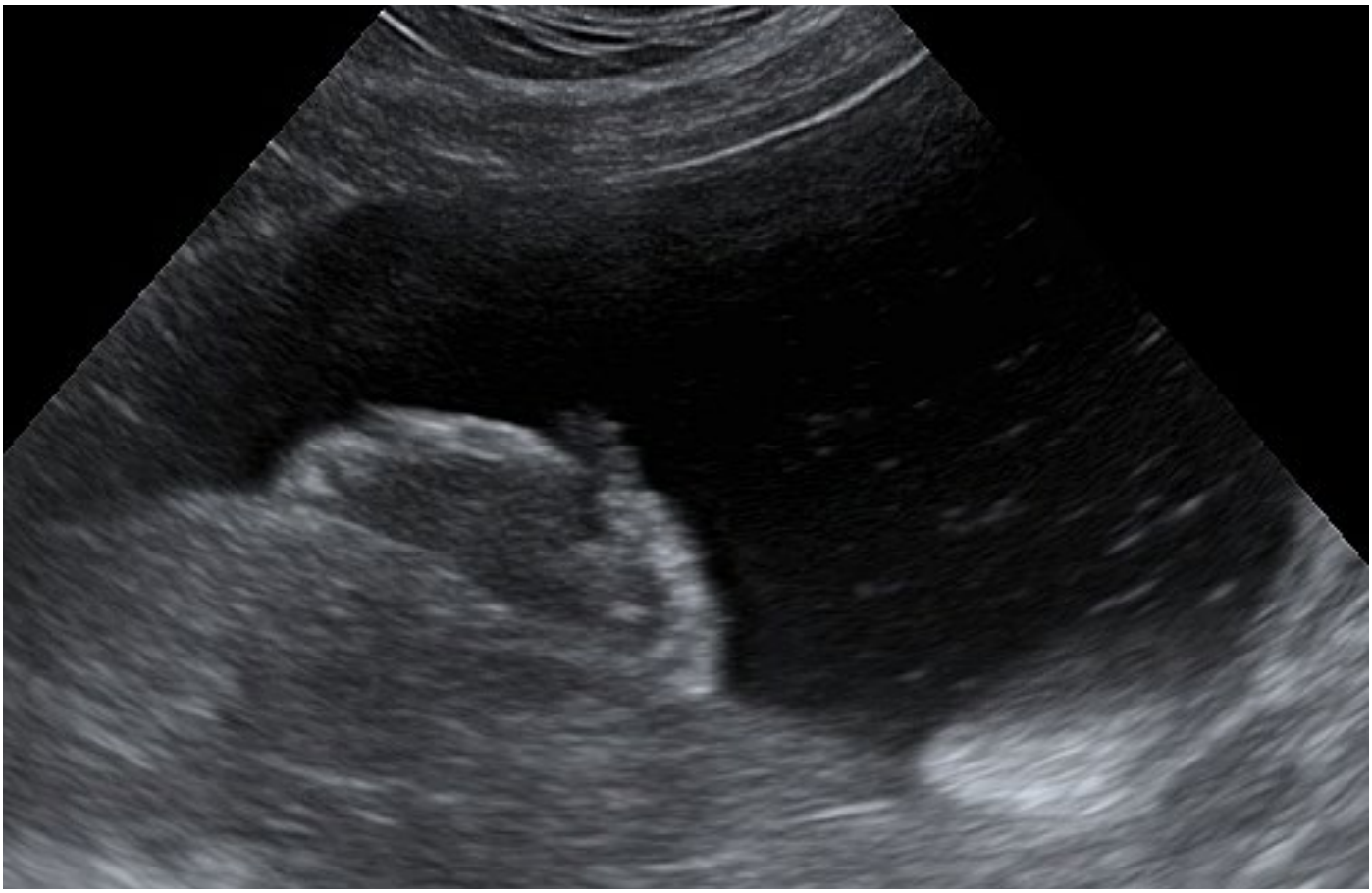

Fig. 1: Ultrasound image of a urinary bladder carcinoma

Image source: Dr. G. Dinges, Kleintierpraxis Wachau

Urothelial and prostate carcinomas

Both urothelial carcinomas (UCa) of the urinary bladder and urethra and prostate carcinoma (PCa) in dogs are highly malignant neoplasms that are often diagnosed relatively late (e.g. by ultrasound Fig. 1a) and have a poor prognosis. By testing for the presence of the V595E mutation in the ***BRAF*** gene, diagnosis can be made early, even in urine sediment.

NEW is a supplementary test that can be used if no BRAF mutation has been found and the sensitivity of the overall examination needs to be increased. This combined test is called **BRAF comp** at Laboklin.

Both tests are presented here.

BRAF^{V595E} mutation

The *BRAF* variant V595E, known from human medicine, was first investigated in 2015 by Mochizuki et al. in many canine tumours. In contrast to humans, in whom the mutation occurs primarily in malignant melanomas, ovarian tumours, thyroid and colorectal carcinomas, Mochizuki et al. (2015) found the mutation in dogs most frequently in urothelial and prostate carcinomas.

The *BRAF*^{V595E} mutation is a somatic mutation in chromosome 16 that can only be detected in tumour cells. This mutation leads to tumour development via permanent activation of the MAP kinase pathway.

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Indications

Testing for the presence of the *BRAF*^{V595E} mutation can be helpful for the following indications:

- Screening for early detection in predisposed breeds (see below)
- Invasive sampling should be avoided by analysing spontaneous urine (sediment).
- Repeated invasive sampling can be avoided in cases with questionable pathohistological and cytological diagnoses (poor sample quality, superimposed images of inflammation and neoplasia).
- Targeted therapy (e.g. Sorafenib; Chon et al. 2024) in selected BRAF-positive cases

Possible sample materials

- Tissue (e.g. biopsies fixed in formalin, at least 5 mm)
- at least 2 cytological smears (e.g. tumour cell-rich FNA, urinary sediment)
- Urine (1 ml urinary sediment, recommendation: spontaneous morning urine)

As the highly sensitive **method** of digital droplet PCR (ddPCR) is used, just 2 tumour cells with *BRAF*^{V595E} mutation are sufficient to confirm the diagnosis of carcinoma.

Methodological limitations

Analyses of routine material for *BRAF*^{V595E} mutation diagnostics over the past 6 years have shown that no DNA could be isolated in about **10 % of the samples**. This mainly occurs when non-centrifuged urine is sent in instead of sediment. As the DNA is present in the cells, a sufficiently high proportion of epithelial cells must be present in the sample to isolate sufficient DNA. In principle, cell-free DNA can also be detected, but this is not an option that should be relied upon when selecting the material.

In rare cases, **inhibitors** can also be the reason why no DNA can be isolated despite a sufficient number of cells.

To detect the *BRAF*^{V595E} mutation, **bacterial overgrowth** in the urine is usually not a problem.

Specificity and sensitivity

The specificity of *BRAF*^{V595E} mutation diagnostics is 100 %, as the BRAF mutation could not be detected in any of the dogs with cystitis, bladder polyps or similar.

Fig. 2: Jack Russell Terriers carry a BRAF mutation in bladder cancer with particularly high frequency
Image source: PD Dr. H. Aupperle-Lellbach

This also applies to prostate carcinomas, as the *BRAF*^{V595E} mutation was not found in benign prostatic hyperplasia, squamous metaplasia or atrophy of the prostate (Mochizuki et al. 2015a).

Depending on the study and dog breed, the sensitivity of detecting the BRAF mutation in UCa is 71 % and 61 % for PCa. Even early stages (dysplasia) of UCa with a BRAF mutation were detectable in one case report (Chamber et al. 2024).

Current analyses of routine material from LABOKLIN over the past 6 years have shown that the proportion of BRAF-positive samples in the submitted material is exceptionally high in certain terrier breeds (Fig. 2), Shetland Sheepdogs and Beagles. However, this says nothing about the sensitivity, as no information is available on whether the negative cases actually had a tumour. However, these observations indicate that mutation is regularly found in samples from these breeds.

Interpretation of the result

Only the **positive result** is conclusive for a carcinoma.
If **no BRAF mutation** is detectable in the sample, the following scenarios are possible:

- There is no UCa / PCa (e.g. polyp, benign hyperplasia).
- No mutated cells are in the sample, but a carcinoma is present. (questionable representativeness of the sample, e.g. cell-poor cytology/urine).
- The *BRAF*^{V595E} mutation does not cause the carcinoma.
- The new CNA test may be helpful (see below).

NEW: BRAF comp.

LABOKLIN has added a further molecular genetic test (CNA) to diagnose bladder carcinomas and create a complete BRAF comp. panel.
The CNA analysis is based on detecting an altered number of specific gene segments.

In principle, structural gene alterations can lead to a loss or multiplication of gene segments (*copy number alteration, CNA*). Fig. 3

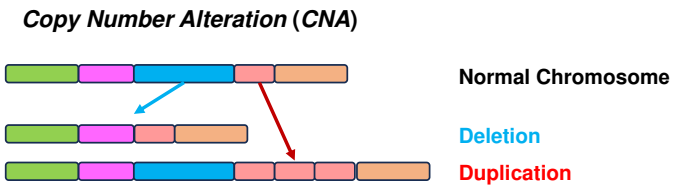


Fig. 3: Schematic representation of possible genetic variations in copy number. *Image source PD Dr. H. Aupperle-Lellbach*

Copy Number Alteration (CNA) beim urothelialen Karzinom des Hundes

Duplications on chromosomes 13 and 36 or deletions on chromosome 19 were found in >75% of cases of canine urothelial carcinoma, with >93% having two or more of these CN alterations (Shapiro et al. 2015). These changes were absent in urine samples from dogs with urinary tract infections, cystitis or benign bladder polyps (Mochizuki et al. 2016).

These gene alterations are NOT detectable in canine prostate carcinomas (see Fig. 4).

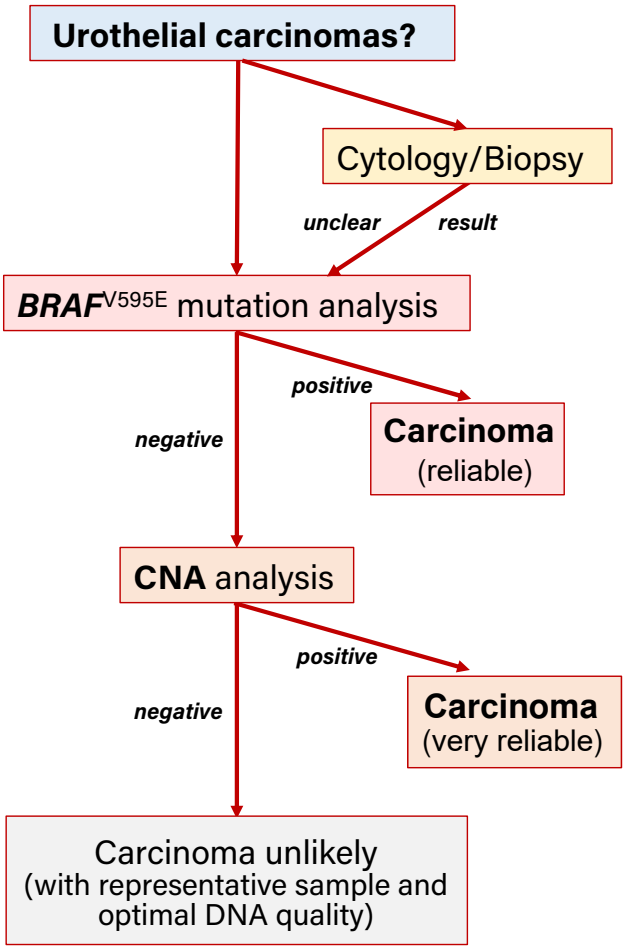
By quantifying these copy numbers, it is now possible to identify urothelial carcinomas that do not have a *BRAF*^{V595E} mutation. The combined test is offered as **BRAF comp.** by LABOKLIN and corresponds to the CADET® BRAF-PLUS test in the USA.

However, the altered copy number is unrelated to the BRAF mutation. Still, it is an independent molecular genetic phenomenon that can only be detected in the tumour cells of canine urothelial carcinomas. The molecular consequences at the protein level or within signalling cascades have not yet been investigated. Whether and to what extent a therapeutic or prognostic statement is possible for dogs with these CNAs in urinary bladder carcinoma remains to be explored.

Methodological limitations

In principle, the material already submitted for *BRAF* mutation analysis (see above) can also be used for CNA analysis.
BUT: Better DNA quality is required, as not only the *BRAF*^{V595E} point mutation is analysed, but also more significant gene segments, which must therefore be available in good quality. There is, therefore, a risk that the CNA analysis will not provide a usable result if the urine is low in cells and/or bacterial overgrowth.

It is therefore recommended that the *BRAF*^{V595E} mutation analysis be performed first and that a subsequent CNA analysis only be considered if the result is negative (see Fig. 3). As the result of the molecular genetic tests is only ever known once the analysis has been fully completed, the total price is due, even if no usable result could be obtained despite repeated testing.



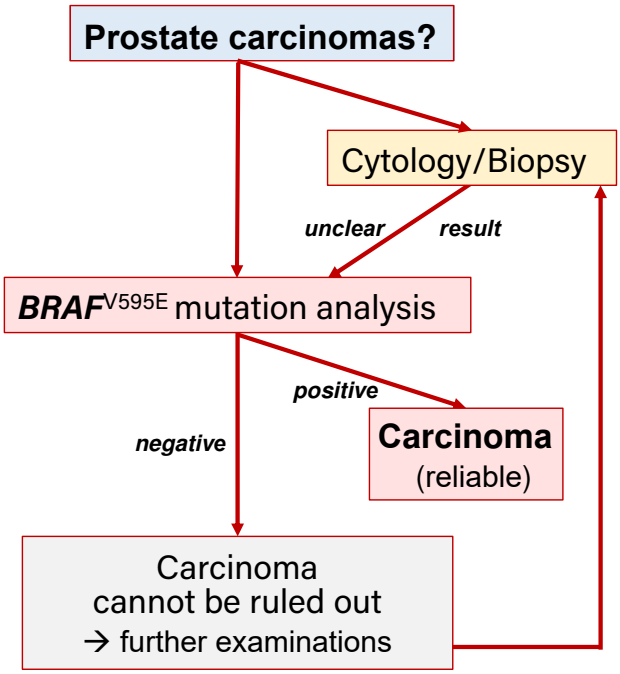


Fig. 4: Diagnostic workflow for the diagnosis of urothelial and prostate carcinomas

Image source: PD Dr. H. Aupperle-Lellbach

Conclusion

Testing for the BRAF mutation is a highly specific method (100 %) for detecting urothelial and prostate carcinomas in dogs. The newly established test for variations in the copy number of specific gene segments (CNA) increased the sensitivity of the molecular genetic diagnosis of urothelial carcinomas.

PD Dr. Heike Aupperle-Lellbach, Alexandra Kehl

Services regarding the topic

- #8675 BRAF mutation (V595E)
- #518 BRAF comp. (V595E + 2 CNA)
- Subsequent request for CNA in case of negative BRAF directly via the laboratory

Further reading

Aupperle-Lellbach H, Grassinger J, Hohloch C et al. Diagnostische Aussagekraft der BRAF-Mutation V595E in Urinproben, Ausstrichen und Bioptaten beim kaninen Harnblasenkarzinom. Tierarztl Prax Ausg K Kleintiere Heimtiere 2018; 46: 289–95.

Aupperle-Lellbach H, Kehl A, Merz S et al. Die BRAF-Mutation V595E im Übergangszellkarzinom – Untersuchungen zur Rassedisposition bei Terriern. Kleintiermedizin 2019; 22(1): 30–3.

Chambers JK, Takahashi N, Kato S et al. Diagnostic challenge in veterinary pathology: Detection of BRAF(V595E) mutation in a dog with follicular cystitis and flat urothelial lesion with atypia. Vet. Pathol. 2024; 61: 335-8.

Chon E, Hendricks W, White M et al. Precision Medicine in Veterinary Science. Vet Clin North Am Small Anim Pract. 2024; 54(3):501-21.

Grassinger JM, Aupperle-Lellbach H, Erhard H et al. Nachweis der BRAF-Mutation bei kaninen Prostataerkrankungen. Tierarztl Prax Ausg K Kleintiere Heimtiere. 2019; 47: 313-20.

Mochizuki H, Kennedy K, Shapiro SG et al. BRAF Mutations in Canine Cancers. PloS one 2015; 10: e0129534.

Mochizuki H, Shapiro SG, Breen M. Detection of BRAF Mutation in Urine DNA as a Molecular Diagnostic for Canine Urothelial and Prostatic Carcinoma. PLoS ONE 2015a; 10, e0144170.

Mochizuki H, Shapiro SG, Breen M. Detection of Copy Number Imbalance in Canine Urothelial Carcinoma with Droplet Digital Polymerase Chain Reaction. Vet. Pathol. 2016; 53: 764–72.

Shapiro SG, Raghunath S, Williams C et al. Canine urothelial carcinoma: Genomically aberrant and comparatively relevant. Chromosome Res. 2015; 23: 311–31.