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The Importance of KRAS Quantification for a Clinicopathological Characterization in Colorectal Cancer Patients

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Abstract: KRAS, a protein whose name stands for Kirsten rat sarcoma, is of practice important nowadays due to its implications in tumorigenesis and metastatic potential. In this paper, the levels of KRAS in colorectal cancer patients have been determined by using a stochastic method and the results have been searched for correlation with clinicopathological features. Patients with their clinical and pathological features were selected from the database of the project GRAPHSENSGASTROINTES and used accordingly with the Ethics committee approval nr. 32647/2018 awarded by the County Emergency Hospital from Targu-Mures. Four kinds of samples have been analyzed (whole blood, saliva, urine, and tissue) by using a stochastic method with stochastic microsensors as screening tools. The results, consisting in levels of KRAS in all the four biological fluids (whole blood, saliva, urine, and tissue), have been correlated with a large series of pathological features such as tumor location among the colon, the tumor dimensions and infiltration depth, gross appearance, budding, stroma features and blood vessels, lymphatic vessels, and perineural invasion. By using KRAS levels in all the four biological fluids, the correlations with clinicopathological features can be extremely useful for the oncologist and the surgeon for a better management of patients. These results are not only extremely valuable, but they can also be obtained in just a couple of hours, with a low cost and high accuracy.

Keywords: colorectal cancer, KRAS, stochastic sensors, cost-effective screening test

1. Introduction

KRAS, standing for Kirsten rat sarcoma, represents a frequently mutated oncogene in colorectal cancer (CRC) (Dienstmann et al., 2020) and its gene mutation is correlated with a poorer prognosis comparing to KRAS wild-type CRC and also with a high probability for the presence of metastases (Dienstmann et al., 2017; Roth et al., 2010). As the upstream KRAS pathway signal regulation is interrupted due to aberrant activation, the result consists in resistance to receptor tyrosine kinase inhibitors, such as cetuximab and panitumumab (Amado et al., 2008; Hong et al., 2020; Karapetis et al., 2008). KRAS gene encodes a guanosine triphosphatase RAS family with a 21 kDa molecular weight whose structure consists of five alpha helices and six beta strands, which form two major domains – a G-domain responsible for the GDP-GTP exchange (Cherfils & Zeghouf, 2013; Simanshu et al., 2017; Vögler et al., 2008) and a hypervariable C-terminal domain (Bourne et al., 1991; Hancock & Parton, 2005). KRAS gene can form two splice variants, namely KRAS 4A and KRAS 4B. The second one is considered to be the main isoform as it is widely found and highly expressed in human cancer, but, recently, it was shown that KRAS 4A is also found in a series of tumors and its function is related to increased adaptability under stress (Chen et al., 2000; Chen et al., 2019; Pells et al., 1997; Tsai et al., 2015).

Cancer development is the result of gene mutations accumulation, which leads to increased cell proliferation (Garraway & Lander, 2013; Stratton et al., 2009; Vogelstein et al., 2013) and, so far, many models predicting the evolution from benign to malign in different tumors have been proposed as the "Vogelgram" for CRC (Fearon & Vogelstein, 1990). The core of this transformation sequence is represented by the KRAS gene mutation, which leads to clonal growth with the expansion of tumor mass (Ionescu, 2004; Kinzler & Vogelstein, 1997) due to

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increase of cell number in lower glucose concentration than normal cells do (Ying et al., 2012; Yun et al., 2009). KRAS gene mutations are found in many cancers such as colon, colorectal, rectum, pancreatic, and lung adenocarcinomas (Buday & Downward, 2008; Hobbs et al., 2016; Pylayeva-Gupta et al., 2011).

In this study, the concentration of KRAS protein in different biological fluids is correlated with clinicopathological features in CRC patients in order to better understand its role in tumorigenesis.

This is not the first time when a study of the influence of KRAS on clinicopathological features is done. Recently, Lee et al. (2020) have studied the differences regarding histology and prognostic in KRAS-mutated colorectal carcinoma. As they mentioned in their study, the results they obtained are consistent with the ones in previous studies. A series of morphological characteristics have been presented and analyzed for wild-type and mutated KRAS mutation status.

It was found that 81.9% of the wild-type KRAS status patients and 82.3% of the mutated KRAS status patients have over 50% infiltrative tumor borders. Regarding degree of differentiation, it was observed that 77.2% of the wild-type KRAS status patients and 79.2% of the mutated KRAS status patients are moderately differentiated while 11.7% of the wild-type KRAS status patients and 15.6% of the mutated KRAS status patients are well differentiated and 11.1% of the wild-type KRAS status patients and 5.2% of the mutated KRAS status patients are poorly differentiated.

Regarding cribriform pattern, 63.2% of the wild-type KRAS status patients and 66.7% of the mutated KRAS status patients have a mild cribriform pattern compound while 12.9% of the wild-type KRAS status patients and 13.5% of the mutated KRAS have no cribriform pattern compound and 24% of the wild-type KRAS status patients and 19.8% of the mutated KRAS status patients have a moderate cribriform pattern compound.

Regarding serrated pattern, 51.5% of the wild-type KRAS status patients and 56.3% of the mutated KRAS status patients have a mild serrated pattern compound while 43.9% of the wild-type KRAS status patients and 30.2% of the mutated KRAS have no serrated pattern compound and 4.7% of the wild-type KRAS status patients and 13.5% of the mutated KRAS status patients have a moderate serrated pattern compound.

The presence and absence of other features have been evaluated – signet ring cells (93.6% of the wild-type KRAS status patients and 96.9% of the mutated KRAS status patients have no signet ring cell), solid component (75.4% of the wild-type KRAS status patients and 82.3% of the mutated KRAS status patients have <50% solid component), papillary component (61.4% of the wild-type KRAS status patients have no papillary component while 54.2% of the mutated KRAS status patients have a papillary component), micropapillary component (81.9% of the wild-type KRAS status patients and 83.3% of the mutated KRAS status patients have no micropapillary component), and tumor-infiltrating lymphocytes (69.6% of the wild-type KRAS status patients and 78.1% of the mutated KRAS status patients have <50% tumor-infiltrating lymphocytes).

Dirty necrosis has been evaluated too depending on quantitative aspects as 6.4% of the wild-type KRAS status patients and 6.3% of the mutated KRAS status patients have no dirty necrosis, 18.7% of the wild-type KRAS status patients and 19.8% of the mutated KRAS status patients have a low presence of dirty necrosis, 29.8% of the wild-type KRAS status patients and 31.3% of the mutated KRAS status patients have a moderate presence of dirty necrosis, 15.2% of the wild-type KRAS status patients and 22.9% of the mutated KRAS status patients have a high presence of dirty necrosis, and 29.8% of the wild-type KRAS status patients and 19.8% of the mutated KRAS status patients have confluent dirty necrosis.

Regarding neutrophilic infiltration, 26.3% of the wild-type KRAS status patients and 13.5% of the mutated KRAS status patients do not associate neutrophilic infiltrate, 32.7% of the wild-type KRAS status patients and 33.3% of the mutated KRAS status patients have low or scattered neutrophilic infiltration, 20.5% of the wild-type KRAS status patients and 21.9% of the mutated KRAS status patients have focal abscesses, and 20.5% of the wild-type KRAS status patients and 21.9% of the mutated KRAS status patients have multiple abscesses (Lee et al., 2020).

Previous studies regarding KRAS have established correlations with clinicopathological features in colorectal patients by using genetic techniques or immunohistochemistry and a large series of results have been obtained (Lee et al., 2020). In our paper, a quantitative and mathematical abording of KRAS has been done. Experimentally, the levels of KRAS in four biological fluids have been determined – whole blood, urine, saliva, and tissue, and the results have been correlated with clinicopathological features from the available database. A series of correlations have been observed as it will be discussed below. Mathematical algorithms for predicting each clinicopathological feature as output have been developed and they can be used by determining the levels of KRAS in the four biological fluids, resembling the inputs.

2. Research Methodology

2.1. Research design: Stochastic method

The detailed stochastic methods and the design of the stochastic sensors used to obtain the concentrations of KRAS in different biological samples were described by Stefan-van Staden et al. (2022; 2023a; 2023b) recently. The signature (toff value) of KRAS is the time needed by KRAS to get into the channel from the membrane of the stochastic sensor; in between to signatures the values of ton (needed in the determination of the concentration of KRAS) are read. The chronoamperometric method was used for the analysis (qualitative and quantitative) of KRAS based on their signatures (the toff values) (Stefan-van Staden et al., 2022; Stefan-van Staden et al., 2023a; Stefan-van Staden et al., 2023b). The quantification of the analyte has been done using the ton values. It was applied a constant potential of 125 mV for the determination of KRAS in the biological fluid. The equations of calibration (1/ton = a + bxCKRAS) related to previous work describing the method design and validation (Stefan-van Staden et al., 2022; Stefan-van Staden et al., 2023a; Stefan-van Staden et al., 2023b) were used for the determination of the concentration of KRAS in biological samples.

2.2. Participants

After the informed consents being received, four kinds of samples were collected from 119 patients confirmed with CRC (110 whole blood samples, 81 saliva samples, 91 urine samples, and 61 tissue samples). The patients were selected from the database of the project GRAPHSENSGASTROINTES and used accordingly with the Ethics committee approval nr. 32647/2018 awarded by the County Emergency Hospital from Targu-Mures.

2.3. Instruments

All chemicals used were of analytical grade. Biomarker extracts were purchased from Sigma Aldrich and paraffin oil (d_4^{20}, 0.86 g/cm^3)

from Fluka. The stochastic sensors were designed and characterized as described earlier.

All the measurements were performed using an Autolab PGSTAT 302 (Metrohm) connected to a computer equipped with the GPES software. The electrochemical cell included the stochastic microsensor, the reference electrode (Ag/AgCl), and the auxiliary electrode (Pt).

3. Results and Discussion

3.1. Location of tumor

The levels of KRAS have determined experimentally in four biological fluids – whole blood, urine, saliva, and tissue – by using the stochastic method presented above. Starting from the obtained values, correlations with clinicopathological features from the available database have been established. In order to get better results, other three parameters have been defined, namely:

$$r_1 = [\mathrm{KRAS}]_{\mathrm{whole\,blood}}/[\mathrm{KRAS}]_{\mathrm{urine}} \quad (1)$$

$$r_2 = [\mathrm{KRAS}]_{\mathrm{whole\,blood}}/[\mathrm{KRAS}]_{\mathrm{saliva}} \quad (2)$$

$$S = [\mathrm{KRAS}]_{\mathrm{saliva}} + 2\bullet[\mathrm{KRAS}]_{\mathrm{urine}} \quad (3)$$

$$S_s = 5\bullet[\mathrm{KRAS}]_{\mathrm{wholeblood}} + 2\bullet[\mathrm{KRAS}]_{\mathrm{urine}} \quad (4)$$

For each feature, cutoff values have been established as given in Table 1.

Regarding tumor location, a series of observations have been done:

- Ascending colon-located tumors associates $S > 20$ μg/ mL, $r_1 > 0.45$, and $r_2 < 0.32$;
- Transverse colon-located tumors associates $S > 20$ μg/ mL, $r_1 < 0.45$, $r_2 > 0.32$, and $[\mathrm{KRAS}]_{\mathrm{whole\ blood}} > 3.5$ μg/ mL;
- Descending colon-located tumors associates $S > 20$ μg/ mL, $r_1 < 0.45$, $r_2 > 0.32$, and $[\mathrm{KRAS}]_{\mathrm{whole\ blood}} > 3.5$ μg/ mL;
- Sigmoid colon-located tumors associates $S < 20$ μg/ mL, $r_1 < 1.4$, $r_2 > 0.32$, and $[\mathrm{KRAS}]_{\mathrm{whole\ blood}} > 3.5$ μg/ mL;
- Sigmorectum junction-located tumors associates S> 14 μg/ mL, $r_1 < 1.4$, $r_2 < 0.8$, $[\mathrm{KRAS}]_{\mathrm{urine}} > 5$ μg/ mL, and $[\mathrm{KRAS}]_{\mathrm{whole\ blood}} < 3$ μg/ mL;
- Rectum-located tumors associate S< 14 μg/ mL, $r_1 < 1.4$, $r_2 > 0.8$, $[\mathrm{KRAS}]_{\mathrm{urine}} < 5$ μg/ mL, and $[\mathrm{KRAS}]_{\mathrm{whole\ blood}} > 3$ μg/ mL.

Regarding tumor budding, the next observations have been done:

- Tumor budding 0 is related to $r_2 < 0.18$;
- Tumor budding 1 is related to $r_2 > 0.18$ and < 0.85;
- Tumor buddings 2 and 3 are related to $r_2 > 0.85$.

Stroma features can be established with high probability from the next observations:

- A predominantly fibrous stroma is associated with $S_s < 25.5$ μg/ mL;
- A mixed stroma is associated with $S_s > 25.5$ μg/mL and $S_s < 75$ μg/ mL;
- A predominantly inflammatory stroma is associated with $S_s > 75$ μg/ mL.

Regarding invasions, the next observations have been done:

Table 1. The cutoff values proposed for each parameter that characterizes the clinicopathological features

Feature		Parameter	Cutoff value (μg/mL)
Location	Ascending colon	S	20
		r_1	0.45
		r_2	0.32
	Transverse colon	S	20
		r_1	0.45
		r_2	0.32
		$[\mathrm{KRAS}]_{\mathrm{whole\ blood}}$	3.5
	Descending colon	S	20
		r_1	0.45
		r_2	0.32
		$[\mathrm{KRAS}]_{\mathrm{whole\ blood}}$	3.5
	Sigmoid colon	S	20
		r_1	1.4
		r_2	0.32
		$[\mathrm{KRAS}]_{\mathrm{whole\ blood}}$	6
	Rectosigmoid junction	S	14
		r_1	1.4
		r_2	0.8
		$[\mathrm{KRAS}]_{\mathrm{whole\ blood}}$	3
		$[\mathrm{KRAS}]_{\mathrm{urine}}$	5
	Rectum	S	14
		r_1	1.4
		r_2	0.8
		$[\mathrm{KRAS}]_{\mathrm{whole\ blood}}$	3
		$[\mathrm{KRAS}]_{\mathrm{urine}}$	5
Tumor budding	1	r_2	0.18
	2	r_2	0.85
	3	r_2	0.85
	4	r_2	0.85
Stroma features	Fibrous	S_S	25
	Mixed	S_S	75
	Inflammatory	S_S	75
Invasions	Blood vessels	$[\mathrm{KRAS}]_{\mathrm{whole\ blood}}$	8.3
		$[\mathrm{KRAS}]_{\mathrm{saliva}}$	10.5
	Lymph vessels	$[\mathrm{KRAS}]_{\mathrm{whole\ blood}}$	3.25 and 8.5
	Perineural	$[\mathrm{KRAS}]_{\mathrm{whole\ blood}}$	1.5 and 13.5

- Blood vessels invasion is related to $[\mathrm{KRAS}]_{\mathrm{whole\ blood}} > 8.3$ μg/ mL or $[\mathrm{KRAS}]_{\mathrm{saliva}} > 10.5$ μg/ mL;
- Lymph vessels invasion is related to $[\mathrm{KRAS}]_{\mathrm{whole\ blood}} > 8.5$ μg/ mL;
- Perineural invasion is related to $[\mathrm{KRAS}]_{\mathrm{whole\ blood}} > 13.5$ μg/ mL.

One of the analyzed features is the location and for this, the colorectal area has been divided into six regions – the ascendant colon (C1), the transverse colon (C2), the descendent colon (C3), the sigmoid

colon (C4), rectosigmoid junction and adjacent area (C5), and rectum (C6).

Each location has been searched using a series of parameters called criteria, which reflects molecular biodynamics and metabolism of KRAS. The first criterion is the whole blood concentration, which reflects the mass transfer of KRAS from the tumor mass to the interstitial adjacent space and into blood. The second criterion evaluates the renal elimination of KRAS and it is represented by the ratio $r_1 = [KRAS]_{whole\ blood}/[KRAS]_{urine}$. The third criterion evaluates the elimination via salivary glands, and it is represented by the ratio $r_2 = [KRAS]_{whole\ blood}/[KRAS]_{saliva}$. The fourth criterion is given by an approximation of the total elimination via kidneys and salivary glands in the assumption that daily output of kidney is twice the salivary glands and it is calculated as $S = [KRAS]_{saliva} + 2 \bullet [KRAS]_{urine}$.

It was observed that higher levels of KRAS in whole blood are linked to C1 and C4 tumors, where 64.28% of the patients (C1) and 56% of the patients (C4) had concentrations over 3 μg/mL.

Regarding r_1 values, lower values were linked to C2 and C3 patients where 66.66% of the patients had values under 0.45. Moreover, higher values of r_1 were linked to C5 and C6 location, where 57.14% (C5) and 62.96% (C6) of the patients had values over 1.4.

By analyzing the third criterion, it was observed that lower values have been associated with C2 and C3 patients as 83.33% of the patients had values of r_2 under 0.32. On the other side, C4 and C6 patients have been linked to higher values as 68.42% (C4) and 79.17% (C6) had values higher than 0.8.

The last parameter discussed is the total elimination criterion. It was observed that lower values have been related to C6 patients as 59.1% of the patients had values < 14 μg/mL and 72.73% of the patients had values < 20 μg/mL. On the other hand, higher values were linked to C1 patients as 85.71% of the patients had levels >20 μg/mL.

Starting from the observations above, groups of criteria values have been elaborated for differentiating different locations. This way, there has been proposed an algorithm for a probabilistic establishment of the location that can predict with high probability the location.

By using the values of S, C1, C2, and C3 patients have differentiated from the others as 85.71% of the C1 and 83.33% of the C2 and C3 patients had values >20 μg/mL compared to only 18.75% of the C4 patients, 28.57% of the C5 patients, and 27.27% of the C6 patients.

Differentiation of C1 from C2 and C3 locations can be done by using the second and the third criteria. By setting a cutoff value of 0.45 for r_1, it was observed that 80% of the C1 patients had values over 0.45 while 66.66% of the C2 and C3 patients had levels under the cutoff value. On the other hand, by setting a cutoff value of 0.32 for r_2, it was observed that 83.33% of the C2 and C3 patients had values under the cutoff value while 66.66% of the C1 patients had levels over the cutoff value. A differentiation of C2 patients from C3 patients can be done by using the first criterion as all the C3 patients had whole blood level <3.5 μg/mL and 66.66% of the C2 patients had whole blood level > 3.5 μg/mL.

After excluding C1, C2, and C3, the next step is differentiating C4 from C5 and C6 and a set of criteria has been proposed in order to do it. The chosen criteria were $[KRAS]_{whole\ blood} < 6$ μg/mL, $r_1 < 1.4$, and $[KRAS]_{urine} > 3$ μg/mL. It was observed that 87.5% of the C4 patients have at least 2 out of 3 criteria compared to only 33.33% of the C5 patients and only 35% of the C6 patients. These criteria can also be used to exclude a C4 location if only one or no criterion is respected.

The differentiation of C5 patients from C6 patients cannot be done with a high probability, but a set of criteria has been proposed for a slight differentiation. The set includes $[KRAS]_{whole\ blood} < 3$ μg/mL, $[KRAS]_{urine} > 5$ μg/mL, $r_2 < 0.8$, and $S > 14$ μg/mL. Using these criteria, 66.66% of C5 patients have at least 2 out of 4 criteria respected compared to only 40% of the C6 patients and also 50% of the C5 patients have at least 3 criteria compared to C6 patients.

3.2. Macroscopic features: Gross aspect and dimensions

Regarding dimensions, maximum depth has been correlated to whole blood KRAS level ($p = 0.035$) at is observed a monotony tendency and higher values are related to higher depth values.

By analyzing the gross aspect, a correlation of KRAS level has been observed in urine samples ($p = 0.0493$) as it can be seen in Figure 1. Higher levels tend to be associated with infiltrative and ulcerative-infiltrative features while lower values are associated with vegetant features.

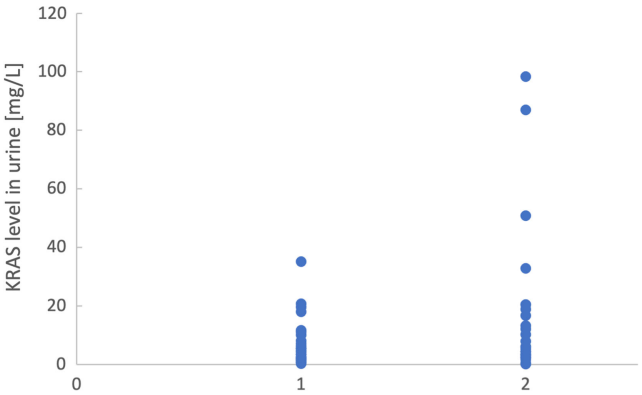

Figure 1. The relationship between KRAS levels and gross features. 1 – vegetant features and 2 – infiltrative features

3.3. Microscopic features: Tumor budding and stroma features

Tumor budding was slightly related to whole blood KRAS levels ($p = 0.065$) as higher levels were linked to higher budding values and to saliva KRAS levels ($p = 0.08$) as lower levels were linked to higher budding values. Starting from these observations, the ratio $r = [KRAS]_{whole\ blood}/[KRAS]_{saliva}$ has been calculated and it is expected that lower values of r to be associated with lower tumor budding values. By setting cutoff values for this ratio, the percentages of patients with respect to the cutoff value have been calculated and the results are given in Table 2.

Table 2. The percentages of patients for each value of tumor budding with respect to the cutoff value

	Tumor budding			
r	0	1	2	3
<0.18	75.00%	22.73%	7.69%	5.88%
<0.85	100.00%	54.55%	23.08%	23.53%
>0.85	0.00%	45.45%	76.92%	76.47%
>1.4	0.00%	31.82%	53.85%	58.82%

It is observed that most patients with tumor budding value of 0 have $r < 0.18$ while most of patients with tumor budding value of 2 and 3 have $r > 0.85$.

Regarding stroma features, which were classified as only inflammatory, fibrous and inflammatory, and only fibrous, it is observed that the presence of fibrous compound is related to lower levels of KRAS in whole blood and urine and a new criterion has been developed, called $S_s = 5 \bullet [KRAS]_{whole\ blood} + 2 \bullet [KRAS]_{urine}$, the quotient being chosen starting from the assumption of a medium value of 5 l of whole blood and 2 l of urinary output in a day. It was obtained that the values of S_s were correlated with stroma features ($p = 0.025$) as smaller values of S_s are correlated with the presence of a fibrous compound. It was observed that all the patients with only a fibrous compound had values of S_s <25.5 μg/mL compared to only 56.1% of the ones with mixed features and only 31.82% of the ones with inflammatory stroma. Moreover, 68.3% of the patients with mixed stroma had S_s values < 35 μg/mL compared to only 36.36% of the ones with inflammatory stroma. On the other side, 36.36% of the patients with only inflammatory stroma had S_s values > 75 μg/mL compared with only 9.76% of the ones with mixed stroma features.

3.4. Microscopic invasions

The last features discussed in this work are the invasions. The lymphatic vessels invasions are correlated with whole blood KRAS levels ($p = 0.00089$) as higher levels are related to lymphatic vessels invasion. By setting as cutoff value 3.25 μg/mL and 8.5 μg/mL, it was observed that levels smaller than 3.25 μg/mL cannot be used for differentiation as 51.85% of the patients with no invasion and 43.14% of the patients with invasion are in this category. By taking into consideration only the patients with KRAS levels in whole blood higher than 3.25 μg/mL, it was observed that 80.77% of the patients with no invasion have $[KRAS]_{whole\ blood}$ <8.5 μg/mL while 62.07% of the patients with invasion have $[KRAS]_{whole\ blood}$ >8.5 μg/mL.

Blood vessels invasion was correlated with KRAS levels in whole blood ($p = 0.00014$) and saliva ($p = 0.047$) as higher whole blood KRAS levels and smaller saliva KRAS levels are related to blood vessels invasion. By setting as criteria $[KRAS]_{whole\ blood}$ >8.3 μg/mL and $[KRAS]_{saliva}$ >10.5 μg/mL, it was observed that 63.63% of the patients with no invasion have none of the criteria while 76.47% of the patients with invasion respect at least one of the two criteria.

Regarding perineural invasion, it was only slightly related to whole blood KRAS levels ($p = 0.0262$) as higher values were related to the presence of perineural invasion. By analyzing KRAS levels, it was observed that all the patients with $[KRAS]_{whole\ blood}$ <1.5 μg/mL had no perineural invasion, while 22.58% of the patients with perineural invasion have $[KRAS]_{whole\ blood}$ >13.5 μg/mL compared to only 9.46% of the ones without perineural invasion.

3.5. Summary

The clinicopathological features described above are emphasized in Figure 2.

Summarizing the data given above, the clinicopathological features of a patient can be easily approximated with high probability by following the next steps:

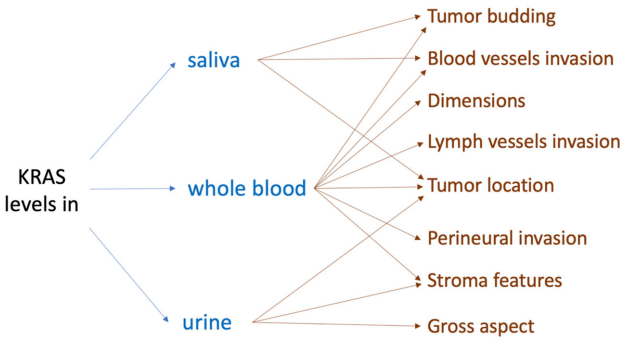

Figure 2. The correlations between the KRAS levels in each biological fluid and the clinicopathological features

a. The location can be anticipating with the criteria and the proposed parameters;
b. The macroscopic features as gross aspect and dimensions can be only qualitative described and they require other biomarkers in order to be mathematically approximated;
c. Tumor budding can be evaluated by using the ratio between KRAS levels in whole blood and saliva;
d. Tumor stroma features can be evaluated by using the values of S_S;
e. The last step involves evaluation of invasions as they are related to levels of KRAS in urine and saliva.

Starting from the steps given above, the entire algorithm can be solved by using coding and the Matlab code proposed as solver is given in Supplementary.

The given code can be easily used in order to approximate the clinicopathological features by only introducing the values obtained for the levels of KRAS.

4. Conclusion

In this paper, a large series of correlations have been obtained between KRAS levels in all the four biological samples (whole blood, saliva, urine, and tissue samples) and pathological features, which can be of use in practice. Criteria and setup of cutoff values for appreciation of tumor location have been elaborated among the colorectal area, the dimensions of tumor, gross features, budding, stroma features and invasions in blood vessels, lymphatic vessel, and perineural one.

The results can be easily obtained by using stochastic sensors, and they can be reached by surgeon and oncologist in only a couple of hours. The results aim to aid the surgeon and the oncologist for a better management of patients.

Conflicts of Interest

The authors declare that they have no conflicts of interest to this work.

Data Availability Statement

The data that support this work are available upon reasonable request to the corresponding author.

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