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From gaps to compliance: a 12-year retrospective cohort study of trends in mismatch repair protein testing and Lynch syndrome identification in colorectal cancer in Central Switzerland

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Summary

STUDY AIM: Alongside an analysis of incidence trends in colorectal cancer and Lynch syndrome over time, the study sought to evaluate the implementation and trends of reflex testing for mismatch repair proteins and key mutations in relevant genes (BRAF, KRAS, NRAS) in colorectal cancer in Central Switzerland from 2011 to 2022, specifically assessing adherence to the Swiss Academy for Quality in Medicine (SAQM) guidelines, in order to identify any gaps or inconsistencies in testing practices that may hinder the diagnosis of Lynch syndrome or microsatellite instability, highlighting areas requiring improvements for optimal patient care.

METHODS: This retrospective study enrolled 2602 patients with 2673 histologically confirmed colorectal cancers. Data collection from the Central Switzerland Cancer Registry included demographic, molecular and immunohistochemical profiles of all histologically confirmed colorectal cancers over the analysed 12-year period. Statistical analyses were performed using R (v4.3.1) with the *tidyverse* package. Normality was assessed with the Shapiro-Wilk test and non-parametric comparisons were made using the Wilcoxon rank-sum test. Chi-square and Fisher's exact tests were used for categorical variables, while Poisson and binomial regression models were used to evaluate temporal trends.

RESULTS: Of 2673 tumours analysed, 76% were tested for mismatch repair proteins, with testing rates improving significantly from 58% in 2011 to >99% in 2022. Among these, 14% showed a mismatch repair protein deficiency, with 77% being MLH1-related and 23% non-MLH1-related, categorising them as Lynch-suspected. 73% (n = 257) of the MLH1-deficient tumours underwent further molecular testing for *BRAF* mutations. Among these, 33% showed no mutation, also categorising them as Lynch-suspected, while the remaining 67% were categorised as sporadic. In total, 6% of the tested tumours were categorised as Lynch-suspected and required further testing and/or genetic counselling. Statistical estimates suggest that among the non-tested tumours, 88 cases could potentially harbour a microsatellite instability, including approximately 5 Lynch-suspected cases. Additionally, in 44 cases, incorrect mismatch repair proteins were tested, potentially leading to missed microsatellite instability. Among the 59 tumours that did not undergo *BRAF* testing, approximately 20 may have been Lynch-suspected and missed due to insufficient testing. Tumour incidence and the proportion of Lynch-suspected tumours among all tumours remained stable over time, without cantonal hotspots.

CONCLUSIONS: Remarkable progress in colorectal cancer diagnostics across Central Switzerland could be demonstrated, leading to a near-complete compliance with guidelines for mismatch repair proteins and molecular testing by 2022. This high adherence to guidelines provides a solid foundation for better personalised surveillance and treatment, ultimately improving the quality of care for colorectal cancer patients in the region. However, during the early years of the study some gaps existed, particularly in testing practices for rectal cancers and incomplete molecular follow-up, potentially missing some patients with a microsatellite instability, who could have benefited from different therapies, and Lynch syndrome patients, who together with their families could have benefited from tighter surveillance.

Introduction

Lynch syndrome, also known as hereditary non-polyposis colorectal cancer (HNPCC), is the most common hereditary cause of colorectal cancer, responsible for approximately 3% of all colorectal cancer cases [1, 2]. It is caused

ABBREVIATIONS

MRPd	mismatch repair protein-deficient
MRPp	mismatch repair protein-proficient
MSI-H	high microsatellite instability
MSI-L	low microsatellite instability

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by inherited autosomal dominant germline mutations in genes encoding mismatch repair proteins (MRPs), which are involved in the repair of deoxyribonucleic acid (DNA) replication errors. Its malfunction results in an accumulation of such errors in areas prone to replication slippage, particularly in repetitive DNA sequences known as microsatellites, resulting in variations in their length. This phenomenon is termed microsatellite instability (MSI) [3]. Rarely, alternative mechanisms can also lead to Lynch syndrome, such as the inactivation of *MSH2* after the 3' terminal deletion of the gene or the constitutive hypermethylation syndrome [4–6].

Microsatellite instability is observed in nearly 15% of colorectal carcinomas and nearly all colorectal tumours associated with Lynch syndrome [7]. Based on the degree of instability, microsatellite status can be classified into three categories: high microsatellite instability (MSI-H), low microsatellite instability (MSI-L) and microsatellite stability. MSI-H tumours typically show significant instability in more than 30% of tested microsatellite markers, depending on the test, with the Bethesda panel being the most commonly used [8]. MSI-H is strongly associated with defective mismatch repair proteins and is frequently observed in Lynch syndrome-associated tumours. MSI-L tumours exhibit a lesser number of instability events, below the 30% threshold, are less clearly associated with Lynch syndrome and their clinical implications are less well-defined in terms of prognosis and treatment response. Microsatellite-stable tumours display no detectable microsatellite instability, indicating an intact mismatch repair mechanism, and represent the majority of colorectal cancer cases.

In daily practice, colorectal cancer can be categorised as either mismatch repair protein-proficient (MRPp) or mismatch repair protein-deficient (MRPd), using immunohistochemistry as a surrogate marker for microsatellite stability or instability. The four main mismatch repair (MMR) genes associated with Lynch syndrome are *MLH1*, *MSH2*, *MSH6* and *PMS2*. Their protein products (MLH1, MSH2, MSH6 and PMS2*)* can be assessed by immunohistochemistry. Mismatch repair protein-deficienttumours can be further categorised into likely somatic or likely syndromic. Somatic tumours arise mainly due to silencing of the *MLH1* gene through methylation of its promoter and are strongly associated with a *BRAF* mutation. Both *BRAF* mutation and *MLH1* methylation analyses are routinely used to confirm the somatic nature of the tumour and rule out Lynch syndrome. Additionally, microsatellite instability can also result as a secondary event due to a DNA polymerase ε *(POLE)* mutation [9]. Occasionally some patients show a so-called "Lynch-like phenotype", which is characterised by a mismatch repair protein deficiency without *BRAF* mutations or promoter hypermethylation, but with somatic double hit mutations instead of a germline mutation [10, 11].

Identifying tumours with a microsatellite instability and individuals with Lynch syndrome is crucial, since the latter show an earlier age of cancer onset and a higher risk for malignancy across multiple organs, mainly colon and endometrium. Additionally, Lynch-associated cancers exhibit distinct prognostic features compared to sporadic cancers [12]. So, these individuals benefit from earlier and more frequent cancer screening and surveillance protocols [13–15].

Some microsatellite-instable tumours belong to the recently introduced hypermutated molecular subtype [16]. The hypermutated nature of these tumours leads to the formation of neoantigens, which in turn trigger an immune response, a feature exploited by immune checkpoint therapies such as pembrolizumab and nivolumab, as they can stimulate the immune system to target cancer cells [17, 18]. Lately, impressive results have been achieved in the treatment of locally advanced rectal cancer [19]; therefore it is now increasingly essential to assess for microsatellite instability, as this can guide an appropriate individualised treatment plan for each patient.

Given the clinical implications of microsatellite instability and Lynch syndrome, the Swiss Academy for Quality in Medicine (SAQM) introduced guidelines in 2011 to improve the detection of mismatch repair protein-deficient tumours and Lynch syndrome in newly diagnosed colorectal cancer [20]. Initially, microsatellite instability testing was recommended only for patients meeting the Revised Bethesda criteria, which included at least one of the following: colorectal cancer before age 50; multiple hereditary non-polyposis colorectal cancer-related cancers; colorectal cancer with microsatellite instability-associated histology before age 60; colorectal cancer / hereditary non-polyposis colorectal cancer in a first-degree relative before age 50; colorectal cancer / hereditary non-polyposis colorectal cancer in at least two first- or second-degree relatives at any age [21]. Testing could be performed either by immunohistochemistry for MLH1, PMS2, MSH2 and MSH6 or by PCR-based microsatellite instability analysis using the Bethesda panel (BAT-25, BAT-26, D2S123, D5S346, D17S250). However, adherence to these criteria was inconsistent, leading to potential underdiagnosis.

To address these limitations, the SAQM updated its guidelines in 2019, implementing universal reflex testing for all newly diagnosed colorectal cancer cases, regardless of clinical criteria [22]. The revised protocol maintained both immunohistochemistry and PCR-based microsatellite instability analysis as standard testing methods.

The Lucerne Cantonal Hospital (LUKS) takes care of a population of around 700,000 [23]. In arithmetical terms, the LUKS therefore manages about 2300 people with Lynch syndrome.

At the Institute of Pathology LUKS, colorectal carcinomas are tested for microsatellite instability according to a standardised algorithm. Immunohistochemistry for mismatch repair protein (MLH1, MSH2, MSH6 and PMS2) is primarily performed on the biopsy specimen. If insufficient or inconclusive, the resection specimen is used. Tumours with retained expression of all mismatch repair protein are classified as MRPp, indicative of microsatellite stability. MRPp carcinomas requiring chemotherapy may be further tested for *BRAF*, *KRAS* and *NRAS* mutations upon clinical request. In contrast, tumours with loss of expression of any mismatch repair protein are classified as MRPd, suggestive of microsatellite instability. Cases with MLH1-loss are reflex-tested mainly for *BRAF* mutation and/or *MLH1* methylation. If a *BRAF* mutation or *MLH1* promoter hypermethylation is identified, a sporadic microsatellite-instable tumour is diagnosed, since these are both strong pre-

dictors of sporadic origin [24]. If neither a *BRAF* mutation nor *MLH1* promoter hypermethylation is detected, the patient is referred for genetic counselling to rule out Lynch syndrome. Younger patients, typically under 50 years old, are recommended for genetic counselling regardless of *BRAF* status [25].

Germline mutation analysis is conducted by geneticists at external institutions, such as the University of Basel or the University of Zurich, or at companies, such as Genetica. The Lucerne Cantonal Hospital offers limited family counselling in cases of diagnosed Lynch syndrome.

The aim of the present study was to evaluate the implementation of reflex testing for mismatch repair protein and mutations in key genes such as *BRAF*, *KRAS* and *RAS* in colorectal cancer in Central Switzerland. It set out to identify any gaps or inconsistencies in testing practices, especially regarding reflex molecular testing for diagnosing or ruling out Lynch syndrome and trends in the implementation of such testing over the 12-year period. The study also compared clinical and demographic characteristics between different cohorts (MRPd vs MRPp, Lynch-suspected vs sporadic) and identified trends in the incidence of colorectal cancer and Lynch syndrome over the years. We expected that the study findings would help to identify any gaps or inconsistencies in testing practices that may hinder the diagnosis of Lynch syndrome or microsatellite instability, highlighting areas requiring improvements for optimal patient care.

Methods

Study setting and design

In total, 2602 patients with 2673 histologically confirmed colorectal carcinomas were enrolled in this retrospective study. A new database was created representing the first comprehensive repository of patients with colorectal carcinomas in Central Switzerland, encompassing not only demographic data but also the molecular and immunohistochemical profiles of the tumours.

Most cases were diagnosed at the Lucerne Cantonal Hospital, for which full access to histological and molecular pathological examination reports were available through the "Pathowin" archive. Pathowin serves as the primary data repository for all histopathological and molecular analyses conducted within the Lucerne Cantonal Hospital Group catchment area. For patients residing in Central Switzerland but treated outside their home canton, data availability relied on mandatory reporting of cancer cases by external clinicians to the Cancer Registry of Central Switzerland.

Inclusion criteria and data collection

The inclusion criteria consisted of individuals residing in Central Switzerland at the time of diagnosis – encompassing the cantons of Lucerne, Obwalden, Nidwalden and Uri – with histologically confirmed adenocarcinomas of the colon, all under ICD-O codes C18.0, C18.2, C18.3, C18.4, C18.5, C18.6, C18.7, C18.8, C18.9, C19.9, C20.9, C21.1 and C21.8 ranging from the caecal pole to the rectoanal junction, as defined by ICD-10 versions 3.1 and 3.2. Metachronous and synchronous carcinomas were included. Only invasive adenocarcinomas stage pT1 or higher according to the definition of the UICC TNM classification were considered. Tumours of the left flexure, descending colon, sigmoid and rectosigmoid carcinomas were classified as left-sided; those of the caecum, ascending colon, right flexure and transverse colon as right-sided; those of the rectum were considered a separate category. Missing or ambiguous data in any category was classified as unknown and excluded from the pertinent calculations.

Data retrieval was conducted both manually and through automated integration from the cancer registry. Manual data extraction from Pathowin provided information on the expression status of the mismatch repair proteins MLH1, PMS2, MSH2 and MSH6 (expressed, not expressed, not tested, unknown); the mutation status of the genes *KRAS*, *BRAF*, *NRAS* (mutated, not mutated, not tested, unknown); the methylation status of the *MLH1* promotor (methylated, not methylated, not tested, unknown); microsatellite instability PCR results (microsatellite stability, MSI-low, MSI-high, not tested, unknown) and type of resected specimen (biopsy, resection specimen). Additionally, data from the cancer registry was automatically pooled including tumour identification number, number of tumours per patient, sex (male, female), incidence age, canton of residence at the time of incidence (Lucerne, Uri, Obwalden, Nidwalden), year of occurrence, ICD-10 and ICD-O location code, ICD-O morphology codes and tumour grade (G1, G2, G3, unknown).

Each registry entry underwent meticulous verification against the original pathological reports to ensure data accuracy. Cases diagnosed within the Lucerne Cantonal Hospital Group allowed for direct cross-validation using Pathowin, ensuring data completeness. However, for externally diagnosed cases, the analysis was restricted to information available from the cancer registry. If histological reports with mismatch repair protein status or molecular reports on mutation status were incomplete or not transmitted by external institutions, these data were not accessible. This limitation primarily affected manually retrieved variables, whereas automated data from the cancer registry remained complete.

To address missing data, we systematically reviewed all available sources, including pathological reports, molecular testing reports, tumour board summaries and oncological records. If data remained unavailable despite these efforts, the respective data fields were classified as "unknown" and excluded from the affected analyses.

The Cancer Registry of Central Switzerland, which provided part of the data, has systematically documented patient data of colorectal cancer since 2010, with validated data extending only until mid-2023 at the time of writing. So, the study analysed the data of all 12 years available – from 2011 to 2022 – in full.

Laboratory methods

At the Lucerne Cantonal Hospital, immunohistochemistry was performed on FFPE samples on a Bond III machine (Leica) following internally established and validated protocols. The following antibodies were used: MLH1 (NCL-L-MLH1, Novocastra), PMS2 (BSB-BSB 2124, BioSB), MSH6 (AC-0047EU, Epitomics) and MSH2 (286M-16,

Cell Marque). The absence of nuclear staining in the presence of internal controls, as well as abnormal staining patterns like dotted patterns or cytoplasmic staining, were interpreted as negative. Mutational analysis for *BRAF*, *KRAS* and *NRAS* was performed using mainly Sanger sequencing (home-made assay) and recently with the Oncomine Precision Assay v3 GX (Thermo Fisher Scientific). The microsatellite instability was analysed with a multiplex PCR assay targeting the Bethesda markers [26]. *MLH1* promoter methylation was assessed with Pyromark Gold Q24 Reagents 5x24 (971802, Quiagen).

Statistical analysis

All statistical analyses were conducted using R (v4.3.1), using the *tidyverse* framework, including *dplyr* (v1.1.4) for data wrangling and *ggplot2* (v3.5.1) for data visualisation. Normality of data was assessed using the Shapiro-Wilk test and a non-parametric test, the Wilcoxon rank-sum test with continuity correction, was employed for non-normally distributed data. A chi-square test was used to compare the distribution of categorical variables between two cohorts, with Fisher's exact test employed for smaller sample sizes. Poisson and binomial regression models were used to evaluate temporal trends in tumour incidence, Lynch tumour frequency and tumour testing proportions. Models estimated yearly changes with 95% confidence intervals; binomial confidence intervals for observed data were calculated using the Wilson method. Data on the permanent resident population by canton per year, as published by the Federal Statistical Office (Bundesamt für Statistik), was used. [27]

Statistical significance was defined as $p < 0.05$. Where not specified, results can be assumed to have reached a significance level of $p < 0.01$.

Ethics approval

The study was approved by the Ethics Committee Northwestern and Central Switzerland (EKNZ).

Results

General patient and tumour characteristics

Cohort characteristics are summarised in table 1. During the analysed period from 2011 to 2022, a total of 2673 tumours were identified in 2602 patients (1533 men and 1069 women). The median age of incidence was 71 (range: 24 to 101). There was no significant difference in tumour incidence between the sexes. No statistically significant difference in incidence was found between cantons.

General testing behaviour and outcomes of mismatch repair protein testing and mutation analysis

Testing behaviour is outlined in figure 1. Among the 2651 tumours with available data, 76% (n = 2017) were tested for at least one mismatch repair protein, leaving 24% (n = 634) that were not tested for mismatch repair proteins. There was no significant difference in patient age between the tested and non-tested tumours.

14% of the tested tumours showed a mismatch repair protein deficiency (MRPd) on immunohistochemistry. Among these, 77% showed loss of MLH1, with concurrent PMS2 loss assumed in cases where only MLH1 was tested, while 23% of cases showed a non-MLH1-related deficiency (figure 1).

Regarding molecular testing, of the 2585 tumours with available data, 34% underwent molecular testing (figure 1): 785 tumours underwent *BRAF* testing, with 22% showing mutations; 734 tumours underwent *KRAS* testing, with 51% showing mutations; and 646 tumours underwent *NRAS* testing, with 3% showing mutations. Interestingly, five MRPp tumours showed concurrent mutations: two had both *BRAF* and *KRAS* mutations, one had *BRAF* and *NRAS* mutations and two had mutations in *BRAF*, *KRAS* and *NRAS*.

Table 1:
Cohort characteristics. All values represent the number of cases (n); unless otherwise specified, percentages in brackets (%) are based on the total number of available cases per category. Missing data is indicated at the bottom of the table.

Descriptive data		Category	n (% of total)
Patients (n = 2602)	Patient age of incidence	Median (range)	71 (24–101)
		<50 years	188 (7%)
	Patient sex	Male	1533 (59%)
		Female	1069 (41%)
	Patient canton of residence	Lucerne	2035 (78%)
		Nidwalden	212 (8%)
		Obwalden	175 (7%)
		Uri	180 (7%)
	Patient tumour count	Single tumour	2537 (98%)
		Multiple tumours	65 (2%)
Tumour count (n = 2673)	Tumour location*	Right colon	923 (35%)
		Left colon	981 (37%)
		Rectum	743 (28%)
	Tumour grading**	Grade 1 (WHO low-grade)	133 (5%)
		Grade 2 (WHO low-grade)	1672 (65%)
		Grade 3 (WHO high-grade)	749 (29%)

* 26 values missing, leaving 2647 values

** 119 values missing, leaving 2554 values

Testing behaviour among the MRPd cohort

Among the 281 MRPd tumours, data was unavailable for 4 cases and excluded from the calculations. Of the remaining 277 tumours, 66% underwent molecular testing. All but one underwent *BRAF* testing, with 64% showing a *BRAF* mutation; among the latter group, 95% had a p.V600E mutation in exon 15, while the remaining mutations were p.D594G and p.K601E, also in exon 15, or remained unknown. Additional *KRAS* testing was carried out for 54 tumours, independently of *BRAF* status, with 19% showing an isolated *KRAS* mutation. Finally, 52 tumours underwent *NRAS* testing, independently of *BRAF* status, with 4% showing an isolated *NRAS* mutation. No concurrent mutations were observed for any of the three analysed genes.

Of the 216 tumours with lost MLH1 expression, only 73% were further tested for *BRAF*. One third of them (n = 52 or 33%) showed no mutation, categorising them as Lynch-suspected, while the remaining two thirds (n = 105 or 67%) showed a *BRAF* mutation, categorising them as sporadic (figure 1).

The remaining 65 cases showed a non-MLH1-related MRPd and were classified as Lynch-suspected (figure 1).

In total, 6% (n = 117) of the 2017 tested tumours were considered Lynch-suspected and required further testing and/or genetic counselling.

Trends over time in tumour incidence, testing rates and Lynch-suspected tumours

Temporal trends in tumour incidence, testing rates and the proportion of Lynch-suspected tumours were assessed over the study period (2011–2022) (figure 2).

Tumour incidence remained stable over the study period. In the Poisson regression model adjusted for population size, no significant association between year and incidence was observed ($\beta = -0.001$, SE = 0.006, p = 0.886), corresponding to an incidence rate ratio of 0.999 per calendar year. A likelihood ratio test comparing a full model including canton to a reduced model excluding canton showed no improvement in model fit ($\chi^2(3) = 0.740$, p = 0.860), indicating no substantial regional variation in incidence rates (figures 2B, 2C).

The proportion of Lynch-suspected tumours among all tumours remained stable over time. The binomial regression model yielded a year coefficient of $\beta = 0.005$ (SE = 0.027, p = 0.862), corresponding to an odds ratio of 1.005 (95% CI: 0.953–1.060) per calendar year. While the proportion in 2018 exceeded the model-predicted range (9%), this deviation was considered likely due to random variation (figure 2D).

In contrast, the proportion of tumours tested showed a significant upward trend. The binomial regression model estimated a year coefficient of $\beta = 0.211$ (SE = 0.015, p <0.001), corresponding to an odds ratio of 1.235 (95% CI: 1.200–1.271) per calendar year. This reflects a substantial annual increase of approximately 24% in the odds of tumour testing throughout the study period (figure 2A).

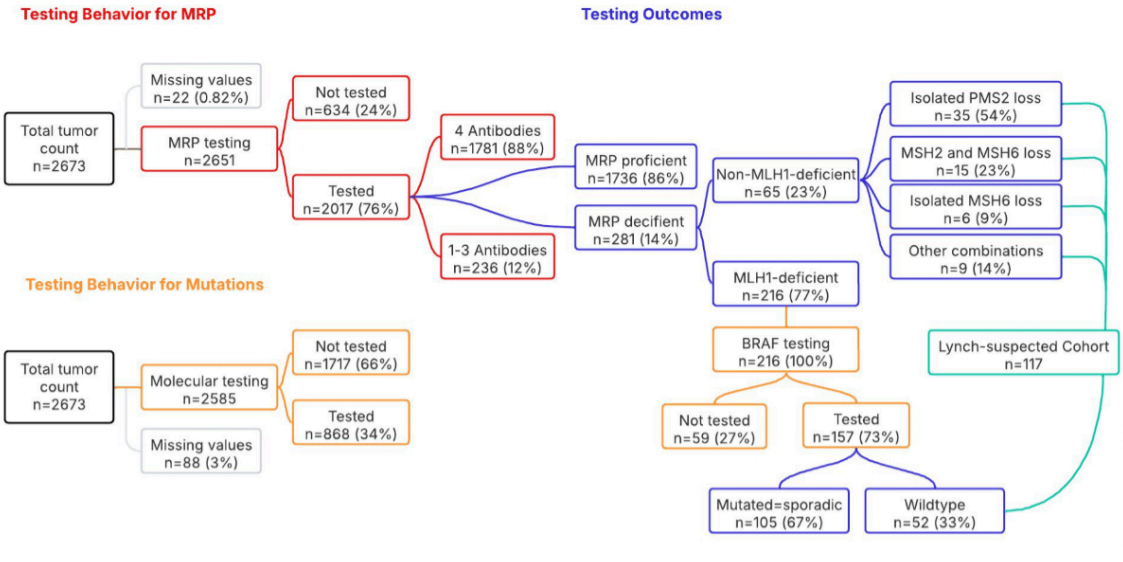
Comparison between the MRPd and MRPp cohorts

Significant differences were found between MRPd and MRPp tumours across several factors, as shown in table 2. The median age of diagnosis was higher for MRPd tumours (76 years) compared to MRPp tumours (70 years). MRPd tumours were more likely to be high-grade (grade 3), were more common in females and were predominantly located in the right colon.

Comparison between the Lynch-suspected cohort and the sporadic cohort

The Lynch-suspected and sporadic cohorts differed in terms of median age (67 vs 78 years, respectively) and minimum age (27 vs 54 years, respectively). Notably, all 18 cases with an incidence age younger than 50 years were

Figure 1: Testing behaviour and outcomes.This flowchart provides an overview of the key results of testing behaviour for mismatch repair protein (MRP) status and mutation analysis of all diagnosed colorectal cancers from 2011 to 2022 in Central Switzerland, along with the main testing outcomes. n represents the number of tumours and the percentage (%) indicates the proportion within the specific parent category.



exclusively observed in the Lynch-suspected cohort (table 2).

Although right-sided tumours predominated in both cohorts, all 9 rectal carcinomas were found exclusively in the Lynch-suspected cohort.

A statistically significant difference in sex distribution was observed (p = 0.017), with a higher proportion of females in the sporadic cohort compared to the Lynch-suspected cohort.

No significant differences were detected in grading or regarding the distribution of cases across cantons, suggesting a lack of geographical clustering or territorial hotspots.

Figure 2: Time trend analysis (2011–2022). A: Proportion of tumours tested for mismatch repair proteins over time, increasing from 0.57 in 2011 to 0.99 in 2022. B: Tumour incidence per 100,000 over time. C: Tumour incidence per 100,000 by canton over time. D: Proportion of Lynch-suspected tumours (%) over time. CI: confidence interval; LU: Lucerne; NW: Nidwalden; OW: Obwalden; UR: Uri.

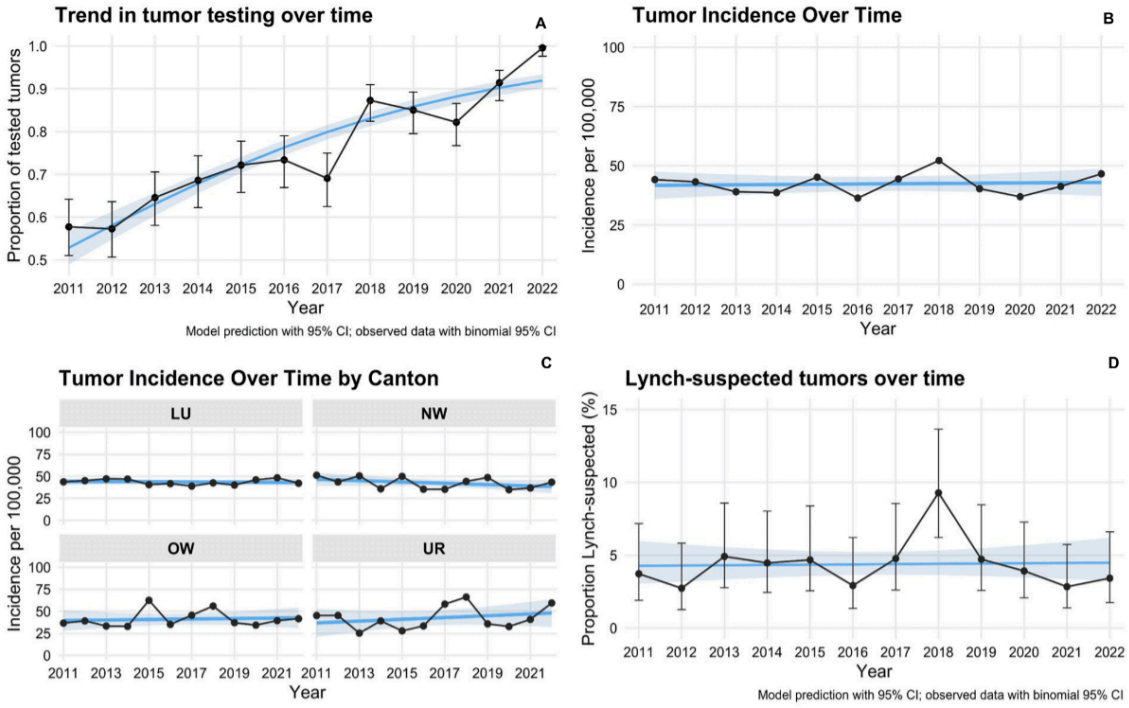


Table 2:
Comparison between the MRPd and MRPp cohorts and between Lynch-suspected and sporadic cohorts. All values represent the number of cases (n); unless otherwise specified, the percentages in brackets (%) are based on the total number of available cases per category. Missing data is indicated at the bottom of the table.

Descriptive data		**MRPd vs MRPp tumours**		**Lynch-suspected vs sporadic tumours**	
		MRPp tumours	**MRPd tumours**	**Lynch-suspected tumours**	**Sporadic tumours**
Tumour count*		1736	281	117	105
Age of incidence	Median (range)	70 (24–101)	76 (27–94)	67 (27–92)	78 (54–94)
	<50 years**	169 (10%)	19 (7%)	18 (15%)	0 (0%)
Sex	Male	1069 (62%)	105 (37%)	55 (47%)	32 (30%)
	Female	667 (38%)	176 (63%)	62 (53%)	73 (70%)
Canton of residence	Lucerne	1322 (76%)	240 (85%)	101 (86%)	93 (89%)
	Nidwalden	146 (8.4%)	19 (7%)	8 (7%)	5 (5%)
	Obwalden	134 (7.7%)	9 (3%)	5 (4%)	3 (3%)
	Uri	134 (7.7%)	13 (5%)	3 (3%)	4 (4%)
Tumour location***	Right colon	566 (33%)	236 (84%)	92 (79%)	94 (90%)
	Left colon	711 (41%)	34 (12%)	16 (14%)	11 (10%)
	Rectum	448 (26%)	10 (4%)	9 (8%)	0 (0%)
Tumour grading****	Grade 1 (WHO low-grade)	98 (6%)	9 (3%)	2 (2%)	4 (4%)
	Grade 2 (WHO low-grade)	1125 (67%)	109 (39%)	46 (40%)	42 (40%)
	Grade 3 (WHO high-grade)	461 (27%)	161 (58%)	68 (58%)	58 (56%)

MRPd: mismatch repair protein-deficient; MRPp: mismatch repair protein-proficient.

* 59 missing values for Lynch-suspected vs sporadic tumours, leaving 222 values.

** 1 missing value for Lynch-suspected vs sporadic tumours, leaving 18 values.

*** 11 missing values for MRPp tumours, leaving 1725 values; 1 missing value for MRPd tumours, leaving 280 values.

**** 52 missing values for MRPp tumours, leaving 1684 values; 2 missing values for MRPd tumours, leaving 279 values; 1 missing value for Lynch-suspected, leaving 116 values; 1 missing value for sporadic tumours, leaving 104 values.

Discussion

This retrospective study provided a comprehensive overview of testing behaviours for mismatch repair protein in all colorectal cancer across Central Switzerland, identifying gaps and non-adherence to published guidelines of the Swiss Academy for Quality in Medicine (SAQM) in reflex testing for mismatch repair protein and, when appropriate, molecular testing. By identifying inconsistencies and highlighting positive trends, we aim to raise awareness among pathologists to improve the identification of patients with Lynch syndrome and those who qualify for immune checkpoint inhibitor (ICI) therapy.

In 2011 the SAQM guidelines recommended mismatch repair protein testing by immunohistochemistry or microsatellite instability testing by PCR according to the revised Bethesda Guidelines [20, 21]. This resulted in testing rates of about 60–70% of the tumours, potentially missing patients with MRPd tumours. Pathologists performing the analysis often lacked the full clinical information needed to apply the revised Bethesda criteria, e.g. family history, so relied on non-established criteria for the decision. For example, since rectal tumours are known to show microsatellite instability in fewer than 10% of the cases, these tumours were significantly less likely to undergo testing, indicating that pathologists may be influenced by tumour location, whereas patient age did not appear to impact testing decisions [22]. In the early days, microsatellite status was performed mainly with the goal of ruling out Lynch syndrome, but nowadays the status is crucial for selecting the appropriate treatment. For example, a recent publication showed a nearly complete response in locally advanced rectal carcinomas treated with ICI therapy [28]. In 2019, the SAQM guidelines were revised, stating that immunohistochemistry analysis of the mismatch repair protein with a 4-antibody panel or assessment of the microsatellite instability status by PCR should be performed for all patients with a newly diagnosed colorectal cancer [22]. This policy shift was strongly reflected in our results. Testing rates went up from 56% in 2011 to over 90% in 2021, achieving an impressive near-complete coverage of >99% in 2022 (figure 2A). This substantial progress represents a major achievement in improving colorectal cancer diagnostic practices and adherence to guidelines across the region.

Despite these improvements, significant gaps remained in testing practices, particularly during the early years of the period analysed. Nearly a quarter of the tumours were not tested for mismatch repair protein. Based on statistical estimates, 14% ($n \approx 88$) of the tumours are potentially MRPd, which were missed due to insufficient testing. Notably 44% of those tumours were located in the rectum, compared to a rectal tumour distribution of only 28% in the entire cohort. This suggests that $n \approx 5$ Lynch-suspected cases (6%) may have been missed due to selective bias, especially in the early years of the study period. Age on the other hand played no role in the decision. In 2017–2018, a 2-antibody approach was taken (PMS2 and MSH6) instead of the usual 4-antibody panel. In 44 cases, incorrect testing for MSH2 and MLH1 instead of the recommended PMS2 and MSH6 was performed, impeding the identification of isolated PMS2 or MSH6 deficiencies, leading to another 2% of cases potentially harbouring microsatellite instability. In 12 cases, other mismatch repair protein combinations were tested, due to already known mutations, technical issues or insufficient tissue. Furthermore, *MLH1* promotor methylation analysis was only performed in 9 of the 52 Lynch-suspected tumours with an MLH1 loss without a *BRAF* mutation. Six of them were found to be methylated, ruling out Lynch syndrome. This in part is to be attributed to the lack of clear established protocols at the institution. A standardised approach could reduce the risk of misclassification and ensure consistent identification of Lynch syndrome cases.

A major point of concern lies in incomplete molecular testing following mismatch repair protein deficiency, especially in the MLH1-negative cohort. Of the 216 tumours with lost MLH1 expression, only 157 were further tested for *BRAF*, meaning that of the remaining 59, statistically seen, approximately 20 of these cases may be Lynch-suspected and could have been missed due to insufficient testing.

On a positive note, a trend towards improvement was observed in *BRAF* testing practices. While in 2011 only 67% and in 2013 even 47% of cases underwent *BRAF* testing, the rate increased to 82% in 2018, reaching 96% in 2022. This progressive improvement shows, once again, a positive improvement in testing practices over time. Additionally, the testing rates in patients under 50 years were higher for both mismatch repair protein and molecular testing. Of the 188 patients <50 years, 87% were tested for mismatch repair protein and 7 of 8 with a MLH1 loss underwent *BRAF* testing. Recently Bläker et al. demonstrated that patients under the age of 50 could still have Lynch syndrome despite a *BRAF* mutation and recommended genetic counselling independently of *BRAF* status [25]. Direct referral for genetic counselling could potentially save costs and time on *BRAF* and methylation analysis for younger patients.

In addition to testing practices, our analysis also revealed insights into demographic and clinicopathological characteristics. While the total tumour count showed a 9% increase over the 12-year study period (from 213 cases in 2011 to 232 in 2022), this trend did not translate into a significant rise in tumour incidence when adjusted for population growth ($\beta = -0.001$, $SE = 0.006$, $p = 0.886$). Also, no statistically significant cantonal hotspot in incidence rates was identified (figure 2C). The proportion of Lynch-suspected tumours among all tumours remained consistently low and stable over time, indicating effective regional screening and no apparent increase in Lynch syndrome-associated tumours in Central Switzerland.

MRPp tumours were more frequent in males, primarily left-sided, of lower histological grade and affected younger people. In contrast, MRPd tumours were more frequent in females, primarily right-sided, of higher histological grade and found in older patients, aligning with several previous publications (table 2) [29–31]. In Lynch-suspected cases, compared to MRPd, both were more common in females, predominantly right-sided and of higher grade. However, both groups differed in patient age, with Lynch-suspected patients being younger. This also aligned as expected with several previous publications [32, 33].

The mismatch repair protein-deficiency incidence in our cohort (14%) aligns well with that of similar studies from other Swiss regions. For example, a study conducted in Basel reported an incidence rate of 15%, which also aligns

with broader European data showing an MMR-deficiency prevalence of approximately 15% in colorectal cancer cases [34]. The global incidence of Lynch syndrome is estimated at about 3%, with some geographical and demographic variations [2, 12]. However, due to limitations in our dataset, particularly the lack of genetic testing reports, we classified cases as suspicious for Lynch syndrome when MLH1 expression was lost without a *BRAF* mutation, or when non-MLH1-related mismatch repair protein deficiencies were present, representing 6% of the cohort. Naturally, this rate seems elevated, as it still includes patients without *BRAF* mutation but with *MLH1* promotor methylation and Lynch-like patients, or even false positives. A comparable Swiss study reported similar rates (6%), supporting the robustness of our findings within the constraints of available data [34]. While our study provides valuable insight into reflex testing practices, we acknowledge that direct comparisons with studies using different inclusion criteria (e.g. genetically confirmed Lynch syndrome cases) should be interpreted with caution.

The retrospective design limited our ability to gather complete data, particularly for cases diagnosed externally, which introduced gaps in the cohort. Although most pathological reports came from the Lucerne Cantonal Hospital, where we had access to most medical records, a minority of cases diagnosed externally introduced gaps in the cohort. Despite mandatory reporting of new cancer cases to the registry, some entries were incomplete, particularly those involving molecular tests conducted after the initial diagnosis (for example after metastasis). Changes in testing practices such as using 2 antibodies vs 4 in some years, may also have introduced some bias. Variable interpretation of immunohistochemistry might have also introduced some bias. Initially a positive staining of >10% was sufficient to classify a tumour as MRPp; however more recent publications suggest that any staining pattern deviating from strong uniform positivity warrants further testing, especially since abnormal patterns are more strongly associated with Lynch syndrome [35, 36]. The clinical records on genetic counselling were also mostly incomplete, making it challenging to categorise the cases as Lynch or sporadic. In certain cases, testing might not have taken place due to perceived low clinical relevance.

While current testing algorithms are designed to detect most Lynch syndrome cases, some remain undetected, including cases with a methylated *MLH1* promoter where constitutive methylation syndrome was not considered, a condition found in approximately 3% of patients with Lynch syndrome [5]. These factors suggest that the true number of Lynch syndrome cases in our cohort may be slightly higher than reported.

In conclusion, the tumour incidence and the proportion of Lynch-suspected tumours remained stable over time, with no evidence of cantonal hotspots. In parallel, colorectal cancer diagnostics in Central Switzerland showed remarkable progress, culminating in near-complete compliance with guidelines for mismatch repair protein and molecular testing by 2022. This high adherence provides a solid foundation for better personalised surveillance and treatment, ultimately improving the quality of care for colorectal cancer patients in the region.

Data sharing statement

Open Science: Due to the retrospective nature of the study, a protocol was not prepared.

Anonymised study data can be shared upon request by contacting the corresponding author. Requests must include a detailed study protocol outlining the intended use of the data.

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References

1. Valle L, Vilar E, Tavtigian SV, Stoffel EM. Genetic predisposition to colorectal cancer: syndromes, genes, classification of genetic variants and implications for precision medicine. J Pathol. 2019 Apr;247(5):574–88. http://dx.doi.org/10.1002/path.5229.
2. Yurgelun MB, Kulke MH, Fuchs CS, Allen BA, Uno H, Hornick JL, et al. Cancer Susceptibility Gene Mutations in Individuals With Colorectal Cancer. J Clin Oncol. 2017 Apr;35(10):1086–95. http://dx.doi.org/10.1200/JCO.2016.71.0012.
3. Li GM. Mechanisms and functions of DNA mismatch repair. Cell Res. 2008 Jan;18(1):85–98. http://dx.doi.org/10.1038/cr.2007.115.
4. Castillejo A, Hernández-Illán E, Rodriguez-Soler M, Pérez-Carbonell L, Egoavil C, Barberá VM, et al. Prevalence of MLH1 constitutional epimutations as a cause of Lynch syndrome in unselected versus selected consecutive series of patients with colorectal cancer. J Med Genet. 2015 Jul;52(7):498–502. http://dx.doi.org/10.1136/jmedgenet-2015-103076.
5. Pinto D, Pinto C, Guerra J, Pinheiro M, Santos R, Vedeld HM, et al. Contribution of MLH1 constitutional methylation for Lynch syndrome diagnosis in patients with tumor MLH1 downregulation. Cancer Med. 2018 Feb;7(2):433–44. http://dx.doi.org/10.1002/cam4.1285.
6. Tutlewska K, Lubinski J, Kurzawski G. Germline deletions in the EPCAM gene as a cause of Lynch syndrome - literature review. Hered Cancer Clin Pract. 2013 Aug;11(1):9. http://dx.doi.org/10.1186/1897-4287-11-9.
7. Tariq K, Ghias K. Colorectal cancer carcinogenesis: a review of mechanisms. Cancer Biol Med. 2016 Mar;13(1):120–35. http://dx.doi.org/10.20892/j.issn.2095-3941.2015.0103.
8. Pawlik TM, Raut CP, Rodriguez-Bigas MA. Colorectal carcinogenesis: MSI-H versus MSI-L. Dis Markers. 2004;20(4-5):199–206. http://dx.doi.org/10.1155/2004/368680.
9. Elsayed FA, Kets CM, Ruano D, van den Akker B, Mensenkamp AR, Schrumpf M, et al. Germline variants in POLE are associated with early onset mismatch repair deficient colorectal cancer. Eur J Hum Genet. 2015 Aug;23(8):1080–4. http://dx.doi.org/10.1038/ejhg.2014.242.
10. Martínez-Roca A, Giner-Calabuig M, Murcia O, Castillejo A, Soto JL, García-Heredia A, et al. Lynch-like Syndrome: Potential Mechanisms and Management. Cancers (Basel). 2022 Feb;14(5):1115. http://dx.doi.org/10.3390/cancers14051115.
11. Rodríguez-Soler M, Pérez-Carbonell L, Guarinos C, Zapater P, Castillejo A, Barberá VM, et al. Risk of cancer in cases of suspected lynch syndrome without germline mutation. Gastroenterology. 2013 May;144(5):926–932.e1. http://dx.doi.org/10.1053/j.gastro.2013.01.044.
12. Win AK, Dowty JG, Reece JC, Lee G, Templeton AS, Plazzer JP, et al.; International Mismatch Repair Consortium. Variation in the risk of colorectal cancer in families with Lynch syndrome: a retrospective cohort study. Lancet Oncol. 2021 Jul;22(7):1014–22. http://dx.doi.org/10.1016/S1470-2045(21)00189-3.
13. Idos G, Valle L. Table 8 [Recommended Surveillance for Individuals with Lynch Syndrome]Seattle: University of Washington; 2021.[Internet][[cited 2025 Mar 18]], Available from

https://www.ncbi.nlm.nih.gov/books/NBK1211/table/hnpcc.T.recommended_surveillance_for_ind/
14. Seppälä TT, Latchford A, Negoi I, Sampaio Soares A, Jimenez-Rodriguez R, Sánchez-Guillén L, et al.; European Hereditary Tumour Group (EHTG) and European Society of Coloproctology (ESCP). European guidelines from the EHTG and ESCP for Lynch syndrome: an updated third edition of the Mallorca guidelines based on gene and gender. Br J Surg. 2021 May;108(5):484–98. http://dx.doi.org/10.1002/bjs.11902.
15. Bhattacharya P, Leslie SW, McHugh TW. Lynch Syndrome (Hereditary Nonpolyposis Colorectal Cancer). StatPearls [Internet]Treasure Island (FL): StatPearls Publishing; 2024.[[cited 2024 Jun 26]], Available from http://www.ncbi.nlm.nih.gov/books/NBK431096/
16. Dunne PD, Arends MJ. Molecular pathological classification of colorectal cancer-an update. Virchows Arch. 2024 Feb;484(2):273–85. http://dx.doi.org/10.1007/s00428-024-03746-3.
17. Llosa NJ, Cruise M, Tam A, Wicks EC, Hechenbleikner EM, Taube JM, et al. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. Cancer Discov. 2015 Jan;5(1):43–51. http://dx.doi.org/10.1158/2159-8290.CD-14-0863.
18. Chang L, Chang M, Chang HM, Chang F. Microsatellite Instability: A Predictive Biomarker for Cancer Immunotherapy. Appl Immunohistochem Mol Morphol. 2018 Feb;26(2):e15–21. http://dx.doi.org/10.1097/PAI.0000000000000575.
19. Cercek A, Lumish M, Sinopoli J, Weiss J, Shia J, Lamendola-Essel M, et al. PD-1 Blockade in Mismatch Repair-Deficient, Locally Advanced Rectal Cancer. N Engl J Med. 2022 Jun;386(25):2363–76. http://dx.doi.org/10.1056/NEJMoa2201445.
20. Qualitaet-Qualitaetsrichtlinien-SGPath_DE_2011.pdf [Internet]. [cited 2024 Nov 12]. Available from: https://www.sgpath.ch/customer/files/231/Qualitaet-Qualitaetsrichtlinien-SGPath_DE_2011.pdf
21. Umar A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Rüschoff J, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst. 2004 Feb;96(4):261–8. http://dx.doi.org/10.1093/jnci/djh034.
22. Qualitaet-QRL-KolonRektum-SGPath-2019.pdf [Internet]. [cited 2024 Nov 12]. Available from: https://www.sgpath.ch/customer/files/231/Qualitaet-QRL-KolonRektum-SGPath-2019.pdf
23. Organisation [Internet]. Luzerner Kantonsspital. [cited 2024 Aug 9]. Available from: https://www.luks.ch/ihr-luks/organisation
24. Parsons MT, Buchanan DD, Thompson B, Young JP, Spurdle AB. Correlation of tumour BRAF mutations and MLH1 methylation with germline mismatch repair (MMR) gene mutation status: a literature review assessing utility of tumour features for MMR variant classification. J Med Genet. 2012 Mar;49(3):151–7. http://dx.doi.org/10.1136/jmedgenet-2011-100714.
25. Age-dependent performance of BRAF mutation testing in Lynch syndrome diagnostics - Bläker - 2020 - International Journal of Cancer - Wiley Online Library [Internet]. [cited 2024 Aug 14]. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/ijc.33273
26. Berg KD, Glaser CL, Thompson RE, Hamilton SR, Griffin CA, Eshleman JR. Detection of microsatellite instability by fluorescence multiplex polymerase chain reaction. J Mol Diagn. 2000 Feb;2(1):20–8. http://dx.doi.org/10.1016/S1525-1578(10)60611-3.
27. Statistik B für. Bilanz der ständigen Wohnbevölkerung nach Kanton, 1991-2022 - 1991-2022 | Tabelle [Internet]. Bundesamt für Statistik. 2023 [cited 2024 Aug 9]. Available from: https://www.bfs.admin.ch/asset/de/26565330
28. Trojan J, Stintzing S, Haase O, Koch C, Ziegler P, Demes M, et al. Complete Pathological Response After Neoadjuvant Short-Course Immunotherapy with Ipilimumab and Nivolumab in Locally Advanced MSI-H/dMMR Rectal Cancer. Oncologist. 2021 Dec;26(12):e2110–4. http://dx.doi.org/10.1002/onco.13955.
29. De Smedt L, Lemahieu J, Palmans S, Govaere O, Tousseyn T, Van Cutsem E, et al. Microsatellite instable vs stable colon carcinomas: analysis of tumour heterogeneity, inflammation and angiogenesis. Br J Cancer. 2015 Jul;113(3):500–9. http://dx.doi.org/10.1038/bjc.2015.213.
30. Vilar E, Gruber SB. Microsatellite instability in colorectal cancer-the stable evidence. Nat Rev Clin Oncol. 2010 Mar;7(3):153–62. http://dx.doi.org/10.1038/nrclinonc.2009.237.
31. Ward R, Meagher A, Tomlinson I, O'Connor T, Norrie M, Wu R, et al. Microsatellite instability and the clinicopathological features of sporadic colorectal cancer. Gut. 2001 Jun;48(6):821–9. http://dx.doi.org/10.1136/gut.48.6.821.
32. Martin S, Katainen R, Taira A, Välimäki N, Ristimäki A, Seppälä T, et al. Lynch syndrome-associated and sporadic microsatellite unstable colorectal cancers: different patterns of clonal evolution yield highly similar tumours. Hum Mol Genet. 2024 Nov;33(21):1858–72. http://dx.doi.org/10.1093/hmg/ddae124.
33. Lynch HT, Lynch PM, Lanspa SJ, Snyder CL, Lynch JF, Boland CR. Review of the Lynch syndrome: history, molecular genetics, screening, differential diagnosis, and medicolegal ramifications. Clin Genet. 2009 Jul;76(1):1–18. http://dx.doi.org/10.1111/j.1399-0004.2009.01230.x.
34. Zumstein V, Vinzens F, Zettl A, Heinimann K, Koeberle D, Flüe M von, et al. Systematic immunohistochemical screening for Lynch syndrome in colorectal cancer: a single centre experience of 486 patients. Swiss Med Wkly. 2016 Apr 24;146(1718):w14315–w14315.
35. Rüschoff J, Schildhaus HU, Rüschoff JH, Jöhrens K, Bocker Edmonston T, Dietmaier W, et al. Testing for deficient mismatch repair and microsatellite instability : A focused update. Pathologie (Heidelb). 2023 Nov;44(S2 Suppl 2):61–70. http://dx.doi.org/10.1007/s00292-023-01208-2.
36. Wang C, Zhang L, Vakiani E, Shia J. Detecting mismatch repair deficiency in solid neoplasms: immunohistochemistry, microsatellite instability, or both? Mod Pathol. 2022 Nov;35(11):1515–28. http://dx.doi.org/10.1038/s41379-022-01109-4.