GE – Portuguese Journal of Gastroenterology

Research Article

GE Port J Gastroenterol 2024;31:338–350
DOI: 10.1159/000535920

Received: June 20, 2023
Accepted: November 23, 2023
Published online: January 29, 2024

Risk Factors in Serrated Pathway Lesions: N-Glycosylation Profile as a Potential Biomarker of Progression to Malignancy

Henrique Fernandes-Mendes[a, b] Catarina M. Azevedo[b, c] Mónica Garrido[d] Carolina Lemos[b, c] Isabel Pedroto[a, b] Salomé S. Pinho[c] Ricardo Marcos-Pinto[a, b] Ângela Fernandes[c]

[a]Department of Gastroenterology, Centro Hospitalar Universitário de Santo António, Porto, Portugal; [b]Instituto de Ciências Biomédicas Abel Salazar, University of Porto, Porto, Portugal; [c]Institute for Research and Innovation in Health (i3S), University of Porto, Porto, Portugal; [d]Department of Gastroenterology, Portuguese Oncology Institute of Porto (IPO Porto), Porto, Portugal

Keywords

Colorectal cancer · Serrated pathway · Serrated lesions · *N*-glycosylation · β1,6-GlcNAc branched *N*-glycans · Biomarker

Abstract

Introduction: The serrated pathway contributes to interval colorectal cancers, highlighting the need for new biomarkers to assess lesion progression risk. The β1,6-GlcNAc branched *N*-glycans expression in CRC cells was associated with an invasive phenotype and with immune evasion. Therefore, this study aims to identify potential risk factors for progression of serrated lesions (SLs) to malignancy, analyzing the *N*-glycosylation profile of epithelial/infiltrating immune cells. ***Methods:*** A retrospective cohort study was performed with data from 53 colonoscopies (48 patients). Sixty-three serrated pathway lesions (SPLs) were characterized based on *N*-glycosylation profile (lectin histochemistry/flow cytometry) and *MGAT5* expression. Statistical analysis was performed to search for associations between the glycoprofile and clinical variables from each patient. ***Results:*** Increased β1,6-GlcNAc branched *N*-glycans expression in epithelial cells is found associated with age ($p = 0.007$ in SPL), smoking ($p = 0.038$ in SL), increased BMI ($p = 0.036$ in sessile serrated lesions [SSL]), and polyp dimensions ≥10 mm ($p = 0.001$ in SL), while increased expression of these structures on immune cells is associated with synchronous CA number ($CD4^+$T cells: $p = 0.016$; $CD8^+$T cells: $p = 0.044$ in SL) and female gender ($p = 0.026$ in SL). Moreover, a lower high-mannose *N*-glycans expression in immune cells is associated with smoking ($p = 0.010$ in SPL) and synchronous CA presence ($p = 0.010$ in SPL). Higher expression of these glycans is associated with female ($p = 0.016$ in SL) and male ($p = 0.044$ in SL) gender, left colon location ($p = 0.028$), dysplasia ($p = 0.028$), and adenocarcinoma ($p = 0.010$). ***Conclusions:*** We identified an association between an abnormal glycoprofile and several clinical risk factors, proposing the *N*-glycosylation profile as a potential biomarker of tumor progression in the serrated pathway. The *N*-glycosylation anatomopathological profile analysis could be further used to decide shorter interval follow-up in patients with SPL.

Ricardo Marcos-Pinto and Ângela Fernandes share last authorship.

karger@karger.com
www.karger.com/pjg

Correspondence to:
Ricardo Marcos-Pinto, ricardomarcospinto@sapo.pt
Ângela Fernandes, angelaf@ipatimup.pt

Fatores de risco das lesões da via serreada: perfil de N-glicosilação como um potencial biomarcador de progressão para malignidade

Palavras Chave
Cancro colorretal · Via serreada · Lesões serreadas · *N*-glicosilação · *N*-glicanos β1,6-GlcNAc ramificados · Biomarcador

Resumo
Introdução: A via serreada contribui para os cancros colorretais de intervalo, destacando a necessidade de novos biomarcadores para determinar o risco de progressão destas lesões. A expressão de β1,6-GlcNAc *N*-glicanos ramificados foi associada a um fenótipo invasivo e a evasão imune. Assim, este estudo tem como objetivo identificar potenciais fatores de risco de progressão das lesões serreadas para malignidade, analisando o perfil de N-glicosilação das células epiteliais/células imunitárias. ***Métodos:*** Foi realizado um estudo retrospetivo com dados de 53 colonoscopias (48 doentes). 63 lesões da via serreada foram caracterizadas segundo o perfil de *N*-glicosilação (histoquímica de lectinas/citometria de fluxo) e expressão de *MGAT5*. A análise estatística foi realizada para encontrar associações entre o perfil de *N*-glicosilação e as variáveis clínicas de cada doente. ***Resultados:*** O aumento da expressão de β1,6-GlcNAc *N*-glicanos ramificados nas células epiteliais encontra-se associado com a idade (p = 0.007 nas SPL), tabagismo (p = 0.038 nas SL), aumento do BMI (p = 0.036 nas SSL), e pólipos com dimensões ≥10 mm (p = 0.001 nas SL), enquanto que o aumento destas estruturas nas células imunitárias está associado com o número de CA síncronos (células $TCD4^+$: p = 0.016; células $TCD8^+$: p = 0.044 nas SL) e o género feminino (p = 0.026 nas SL). Além disso, uma diminuição da expressão de N-glicanos ricos em manose está associada ao tabagismo (p = 0.010 para SPL) e a presença de adenomas síncronos (p = 0.010 nas SPL). A expressão aumentada destas estruturas está associado com o género feminino (p = 0.016 nas SSL), género masculino (p = 0.044 nas SSL), localização no cólon esquerdo (p = 0.028), displasia (p = 0–028) e adenocarcinoma (p = 0.010). ***Discussão/Conclusão:*** Identificámos uma associação entre um perfil de glicosilação anormal e vários fatores de risco clínicos, propondo o perfil de *N*-glicosilação como um potencial biomarcador de progressão tumoral na via serreada. A análise anatomopatológica do perfil de *N*-glicosilação pode vir a ser usada para decidir intervalos de follow-up mais curtos em doentes com SPL.

Introduction

Colorectal cancer (CRC) is the third most frequent cancer and it is responsible for 10% of cancer mortality worldwide [1, 2]. This cancer type occurs mostly from conventional adenoma (CA)-carcinoma pathway, while serrated pathway is responsible for about 25% of the cases [3]. Serrated lesions (SLs) are known as the precursor lesion in this carcinogenic pathway. SL can be divided into hyperplastic polyps (HPs), sessile serrated lesions (SSLs), sessile serrated lesions with dysplasia (SSLs-D), traditional serrated adenomas, and unclassified serrated adenomas, accordingly to the World Health Organization [4].

The last two decades were marked by advances in the study of the serrated pathway in order to understand the neoplastic mechanisms underlying this disease to prevent the progression to cancer [5]. This pathway is characterized by epigenetic alterations with mismatch repair genes deficiency and by the presence of a CpG island hypermethylation phenotype, with microsatellite instability in the vast majority of the cases [1, 6, 7]. Consequently, the serrated pathway presents a high lymphocytic immune infiltrate and upregulation of immune checkpoints associated with tumor immune evasion [8]. However, there is a gap of knowledge in understanding this pathway, particularly the progression to malignancy and the risk factors involved.

Some association studies defined smoking, alcohol consumption, overweight, red meat consumption, hypertension, and hypertriglyceridemia as risk factors for the SL development. Other researchers identified aging, absence of regular consumption of non-steroidal anti-inflammatory drugs, polyp dimensions ≥10 mm, dysplasia, female gender, and synchronous CA as risk factors for progression to malignancy in the serrated pathway [7, 9–15]. However, these risk factors are not as well established as in the adenoma-carcinoma pathway. Additionally, this CRC subtype is one of the responsibilities for the occurrence of interval cancers. This is presumed to be secondary to the difficult endoscopic identification of these lesions due to their sessile morphology, mucus coverage, and proximal colon location and rapid cancer progression after the development of dysplasia [7, 9, 10].

Thus, the protective effect of CRC screening is expected to decrease in these patients [7, 10]. This represents a challenge to physicians managing these cases due to the lack of biomarkers that could impact therapeutic decisions. Taking this into account, it seems crucial to identify a new biomarker capable of improving risk stratification and further clinical decision.

N-glycosylation has been associated with the malignant transformation process, and it is considered to be a cancer hallmark [11]. This process is a post-translational modification characterized by enzymatic reactions that allow the binding of carbohydrates (glycans) to proteins, lipids, or other saccharides [11]. These glycan structures are found on all cell surfaces, constituting the glycocalyx [12]. The differential glycans profiles are associated with immunologic and epithelial biologic functions [13]. In fact, our group described that the expression of β1,6-GlcNAc branched *N*-glycans in the conventional colorectal carcinogenesis cascade is considered an important immune checkpoint, demonstrating that these complex *N*-glycans overexpression in CRC cells was associated with immune escape [14]. Additionally, Demetriou et al. [15] demonstrated that T-cell activity is particularly regulated by β1,6-GlcNAc branched *N*-glycans on the T-cell receptor that modulates the threshold of T-cell activation and signaling. In line with this and in the context of chronic inflammatory processes such as inflammatory bowel disease, our group showed that these complex *N*-glycans are capable of regulating T-cell-mediated immune response associated with disease severity [16]. Particularly, we demonstrated that a β1,6-GlcNAc branched *N*-glycans deficiency, due to a *MGAT5* decreased expression, confers an hyperimmune response by decreasing T-cell activation threshold, increasing proinflammatory cytokines production, and increasing T-cell signaling [16]. This highlights the crucial role of the *N*-glycans pattern on cancer development/progression and immune response regulation. Therefore, in this study, we aimed to identify risk factors for progression to malignancy of serrated pathway lesions (SPLs) based on the *N*-glycosylation profile of both cancer cells and infiltrating immune cells.

Methods

Cohort Characterization

This is retrospective cohort study of patients with lesions of the serrated pathway, followed between September 2014 and 2021. Data were collected from 53 colonoscopies, corresponding to 48 patients.

The *N*-glycosylation profile was previously obtained from FFPE (formalin-fixed paraffin-embedded) biopsies and fresh biopsies of SPL. The *MGAT5* gene (gene that encodes the enzyme *N*-acetylglucosaminyltransferase-V [GnT-V]), responsible for the expression of β1,6-GlcNAc branched *N*-glycans, was evaluated by reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR) in FFPE biopsies. Also, in FFPE biopsies, a lectin histochemistry was performed to evaluate the expression of β1,6-GlcNAc branched *N*-glycans (complex glycans) in epithelial and stromal cells, obtained by staining with *Phaseolus vulgaris leucoagglutinin* (L-PHA), as well as the presence of high-mannose *N*-glycans (simple glycans), identified by labeling *Glanthus nivalis agglutinin*. The lectin histochemistry evaluation was performed by two independent observers, who gave a score from 0 to 3 according to the degree of staining (0: ≤25%; 1: 26% to 50%, 2: 51% to 75%, and 3: >75%). Flow cytometry of epithelial cells ($CD45^-$ cells), $CD4^+$ T cells, $CD8^+$ T cells, and $FoxP3^+CD25^+$ T cells (regulatory T cell – Treg) was performed on fresh biopsies, and L-PHA and *Glanthus nivalis agglutinin* expression was obtained, corresponding to β1,6-GlcNAc branched *N*-glycans and high-mannose *N*-glycans expression, respectively, in each of these cell populations.

Statistical Analysis

Descriptive statistics were performed based on the analysis of the mean and standard deviation of the continuous variables under study; percentages were used for the categorical variables. The relationship between the clinical variables and the *N*-glycosylation profile of the epithelial cells and colonic T cells obtained by RT-qPCR, lectin histochemistry, and flow cytometry was performed using Pearson's correlation, *t* test for independent samples, and nonparametric Mann-Whitney U. This relationship was performed for 3 groups: SPL (which includes SL and serrated pathway adenocarcinoma), SL (which includes HP, SSL, and SSL-D), and SSL (with or without dysplasia). The association between continuous variables and non-binary discrete variables was performed using the one-way ANOVA test, using Tukey's post hoc test. A significance level of 0.05 was considered and the statistical analysis of the variables was performed using the SPSS version 26 program.

Results

Of the 53 colonoscopies performed, 63 samples of SPL were obtained (34 FFPE biopsies and 29 fresh biopsies). This cohort includes 44 (69.8%) SSL without dysplasia, 9 (14.3%) SSL-D, 7 (11.1%) HP, 1 (1.6%) adenocarcinoma of the serrated pathway, and 2 (3.2%) SSL-D with concomitant adenocarcinoma (Table 1). The description of the sample regarding the remaining sociodemographic characteristics, risk factors, and anatomopathological characteristics is depicted in detail in Table 1. The descriptive statistic of *N*-glycosylation profile is depicted in detail in Table 2.

Altered Premalignant Epithelial N-*Glycosylation Profile Is Correlated with Age, Smoking, Increased BMI, Polyp's Dimensions, and Lesion Location in the Serrated Pathway*

The *N*-glycosylation profile of epithelial cells was correlated with potential risk factors for disease progression in the serrated pathway. Our results indicated a

Table 1. Cohort characterization

Variable	*N* (%)	Mean	Standard deviation
Gender	48		
Male	24 (50.0)		
Female	24 (50.0)		
Age, years		64.2	11.4
Smoking	48		
No	25 (52.1)		
Yes	23 (47.9)		
Missing	0 (0)		
BMI, kg/m^2	42	27.3	4.9
Lesion number		3.6	4.5
SPL number		3.1	4.4
Synchronous CA number		0.6	1.2
Synchronous CA			
No	47 (74.6)		
Yes	16 (25.4)		
Classification	63		
SSL without dysplasia	44 (69.8)		
SSL-D	9 (14.3)		
HP	7 (11.1)		
Adenocarcinoma	1 (1.6)		
Adenocarcinoma + SSL-D	2 (3.2)		
Location	63		
Right colon	41 (65.1)		
Transverse colon	15 (23.8)		
Left colon	7 (11.1)		
Missing	0 (0)		
Dimension, mm	63	22.8	11.4
<10	4 (6.3)		
≥10	58 (92.1)		
Missing	1 (1.6)		

BMI, body mass index; CA, conventional adenoma; HP, hyperplastic polyp; SPL, serrated pathway lesion; SSL, sessile serrated lesion; SSL-D, sessile serrated lesion with dysplasia.

significant correlation between increased age and the β1,6-GlcNAc branched *N*-glycans expression (L-PHA expression) on epithelial cells in SPL ($p = 0.007$; $r = 0.501$) (Table 3). Additionally, tobacco consumption was also associated with an increased *MGAT5* gene expression. In fact, smokers presented an evident higher β1,6-GlcNAc branched *N*-glycans expression in SL comparing to non-smokers (Δct value: $2.50 \times 10^{-4} \pm 2.62 \times 10^{-4}$ vs. $9.49 \times 10^{-5} \pm 9.82 \times 10^{-5}$; $p = 0.038$) (Table 4). Furthermore, a statistically significant correlation was also observed between body mass index (BMI) and β1,6-GlcNAc branched *N*-glycans expression in SPL ($p = 0.045$; $r = 0.432$) and in SSL ($p = 0.036$; $r = 0.496$), regarding *MGAT5* gene expression (Table 4). In addition, our data indicate a higher β1,6-GlcNAc branched *N*-glycans expression in SL with dimensions ≥10 mm, comparing to SL with dimensions <10 mm, regarding *MGAT5* gene expression (Δct value: $1.70 \times 10^{-4} \pm 1.68 \times 10^{-4}$ vs. $4.40 \times 10^{-5} \pm 3.96 \times 10^{-6}$; $p = 0.001$) (Table 4). Concerning the SPLs location, there was an increase in the high-mannose *N*-glycans expression in the epithelial part of the left colon, compared to the right and transverse colon (2.50 ± 0.71 vs. 0.80 ± 0.84 vs. 0.40 ± 0.22; $p = 0.009$) (Table 4).

Altered Immune N-*Glycosylation Profile Is Correlated with Increased Synchronous CA, Sex, Smoking, Location of the Lesion, and BMI in the Serrated Pathway*

The *N*-glycosylation profile of immune cells was correlated with potential risk factors for disease progression in the serrated pathway. Our results demonstrated a positive correlation between the synchronous CA number in patients with SPL, SL, and SSL and β1,6-GlcNAc branched *N*-glycans expression in CD4$^+$ T cells ($p = 0.022$, $r = 0.475$; $p = 0.025$, $r = 0.477$; $p = 0.044$, $r = 0.493$) and CD8$^+$ T cells ($p = 0.014$, $r =$

Table 2. Statistical description of the glycosylation profile of SPLs

	Mean±standard deviation	Median	Minimum	Maximum
MGAT5 gene RT-qPCR (Δct value)	$2.6 \times 10^{-4} \pm 4.4 \times 10^{-4}$	9.30017×10^{-5}	1.14743×10^{-5}	2.37524×10^{-3}
Histochemistry				
Epithelial L-PHA	1.2±0.9	1.25	0	2
Stromal L-PHA	0.9±0.6	1	0	2
Epithelial GNA	0.9±0.9	0.5	0	3
Stromal GNA	1.6±0.9	1	0.5	3
FC				
Epithelial L-PHA	233.1±156.9	185	20.6	713
Epithelial GNA	148.4±129.9	109	25.7	551
T $CD4^+$ L-PHA	984.4±805.0	838.5	134	3,497
T $CD4^+$ GNA	132.1±146.6	67.5	0	547
T $CD8^+$ L-PHA	1,376.8±1,076.3	1,049	413	4,416
T $CD8^+$ GNA	558.2±965.8	73.2	0	2,843
FoxP3 T $CD25^+$ L-PHA	1,906.7±1,960.7	1,742.5	223	8,775
FoxP3 T $CD25^+$ GNA	1,172.3±2,138.0	559	50.1	9,862

FC, flow cytometry; GNA, *Glanthus nivalis agglutinin*; L-PHA, *Phaseolus vulgaris leucoagglutinin*; RT-PCR, reverse transcriptase-quantitative polymerase chain reaction.

0.602; $p = 0.008$, $r = 0.655$; $p = 0.005$, $r = 0.727$) (Table 3). Furthermore, synchronous CA in patients with SPL was associated with a lower high-mannose *N*-glycans expression in the stromal cells of SPL (0.75 ± 0.25 vs. 1.83 ± 0.94; $p = 0.010$) and specifically in $CD4^+$ T cells in patients with SSL (44.70 ± 13.82 vs. 134.44 ± 157.42; $p = 0.025$) (Tables 3, 4). Furthermore, smokers showed, on average, a lower high-mannose *N*-glycans expression in the SPL stromal cells, when compared with non-smokers (1.19 ± 0.73 vs. 2.67 ± 0.58; $p = 0.010$) (Table 4). Regarding the BMI, we observed an inverse relationship between this factor and the expression of high-mannose *N*-glycans in Tregs in SPL and SL ($p = 0.034$, $r = -0.487$; $p = 0.042$, $r = -0.484$) (Table 3). Additionally, females presented, on average, a higher high-mannose *N*-glycans expression in $CD4^+$ T cells in SPL (178.86 ± 164.04 vs. 67.98 ± 50.69; $p = 0.020$), in SSL (299.87 ± 179.86 vs. 44.08 ± 29.48; $p = 0.017$), and in SL (178.86 ± 164.04 vs. 52.65 ± 38.99; $p = 0.016$) comparing to males (Table 3). Moreover, females showed, on average, a higher β1,6-GlcNAc branched *N*-glycans expression on Tregs in SPL (2,556.36 ± 2,269.88 vs. 1,036.99 ± 990.22; $p = 0.040$), in SL (2,736.70 ± 2,308.10 vs. 1,036.99 ± 990.21; $p = 0.026$), and in SSL (3,005.86 ± 2,759.88 vs. 916.18 ± 813.00; $p = 0.029$), comparing with males (Table 3). On the other hand, males presented, on average, a higher high-mannose *N*-glycans expression in $CD8^+$ T cells in SPL (778.79 ± 1,084.28 vs. 67.98 ± 50.69; $p = 0.044$), in SSL (884.16 ± 1,112.12 vs. 65.55 ± 57.13; $p = 0.043$), and in SL (778.86 ± 1,084.28 vs. 67.78 ± 50.69; $p = 0.044$) (Table 3). Furthermore, left colon lesions presented a higher high-mannose *N*-glycans expression in stromal cells compared to the transverse colon (3.00 ± 0.00 vs. 1.20 ± 0.57; $p = 0.039$) (Table 4). SPL with dysplasia showed a higher high-mannose *N*-glycans expression in stromal cells (2.17 ± 0.98 vs. 0.96 ± 0.33; $p = 0.028$), comparing with non-dysplastic SPL (Table 4). Also, in serrated pathway adenocarcinomas and SSL-D with concomitant adenocarcinoma a higher high-mannose *N*-glycans expression on stroma was observed on average (2.67 ± 0.80 vs. 1.19 ± 0.73; $p = 0.010$) (Table 4).

Discussion

SPL follow-up still raises serious concerns due to rapid progression from dysplasia to cancer. Moreover, there are few robust studies of clinical progression risk factors on serrated pathway. The changes in *N*-glycosylation has been considered as a CRC progression hallmark in epithelial cells [11]. Thus, the main goal of our study was to define potential risk factors for progression to malignancy by analyzing the *N*-glycosylation profile of the serrated pathway.

We found an increased β1,6-GlcNAc branched *N*-glycans expression in the SPL epithelial component correlated with increasing age and BMI. In SL, β1,6-GlcNAc branched *N*-glycans expression in the

GE Port J Gastroenterol 2024;31:338–350
DOI: 10.1159/000535920

Table 3. N-glycosylation profile results obtained by FC

Variable	Epithelial L-PHA			Epithelial GNA			T CD4+ L-PHA			T CD4+ GNA			T CD8+ L-PHA			T CD8+ GNA			Treg L-PHA			Treg GNA		
	N	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value
Gender																								
SPL			0.308			0.443			0.396			**0.014**			0.309			**0.044**			**0.040**			0.980
Male	13	204.62± 90.35		13	139.35± 86.77		13	830.54± 392.51		**14**	**52.65± 38.99**		10	1,182.60± 432.40		**12**	**778.79± 1,084.28**		**13**	**1,036.99± 990.21**		13	1,028.22± 2,679.55	
Female	15	259.87± 171.95		14	176.89± 152.10		13	1,073.85± 935.95		**14**	**178.86± 164.04**		9	1,608.78± 1,204.08		**10**	**67.98± 50.69**		**11**	**2,556.36± 2,269.88**		12	1,049.29± 886.34	
SL			0.587			0.386			0.404			**0.016**			0.195			**0.044**			**0.026**			0.907
Male	13	204.62± 90.35		13	139.35± 86.77		13	830.54± 392.51		**14**	**52.65± 38.99**		10	1,182.60± 432.40		**12**	**778.79± 1,084.28**		**13**	**1,036.99± 990.21**		13	1,028.22± 2,679.55	
Female	14	227.50± 122.14		13	183.19± 156.41		12	1,079.83± 977.31		**13**	**185.30± 168.89**		8	1,739.25± 1,217.31		**10**	**67.98± 50.69**		**10**	**2,736.70± 2,308.10**		11	1,128.50± 883.94	
SSL			0.822			0.369			0.309			**0.017**			0.119			**0.043**			**0.029**			0.958
Male	11	223.76± 77.05		11	148.80± 87.38		11	820.09± 322.43		**12**	**44.083± 29.48**		9	1,161.11± 452.93		**11**	**844.16± 1,112.12**		**11**	**916.18± 813.00**		11	1,138.10± 2,916.71	
Female	11	233.64± 121.67		11	200.48± 164.97		9	1,187.78± 114.37		**11**	**200.37± 179.86**		7	1,892.14± 1,229.08		**8**	**65.55± 57.13**		**7**	**3,005.86± 2,759.88**		9	1,083.39± 918.62	
Age, years																								
SPL	**28**	**0.501***	**0.007**	27	−0.107*	0.596	26	0.206*	0.314	28	−0.183*	0.352	19	−0.101*	0.682	22	0.136*	0.547	24	0.167*	0.436	25	−0.067*	0.751
SL	**27**	**0.422***	**0.028**	26	−0.080*	0.697	25	0.212*	0.308	27	−0.182*	0.363	18	−0.028*	0.913	22	0.136*	0.547	23	0.218*	0.317	24	−0.041*	0.848
SSL	22	0.224*	0.316	22	−0.227*	0.309	20	0.224*	0.343	23	−0.166*	0.449	16	−0.058*	0.830	19	0.104*	0.672	18	0.242*	0.334	20	0.002*	0.993
Smoking																								
SPL			0.195			0.773			0.281			0.715			0.763			0.629			0.357			0.179
No	14	269.07± 143.48		14	151.97± 105.63		13	1,106.08± 922.12		14	106.28± 126.00		11	1,439.00± 1,050.68		11	263.58± 656.57		11	1,349.36± 1,043.60		12	475.91± 721.11	
Yes	14	199.26± 133.45		13	166.19± 145.55		13	798.31± 400.64		14	125.23± 144.70		8	1,309.50± 657.32		11	547.81± 1,058.95		13	2,057.31± 2,294.65		13	1,557.49± 2,610.63	
SL			0.397			0.847			0.287			0.737			0.622			0.629			0.420			0.210
No	13	234.92± 67.93		13	156.35± 108.61		12	1,114.75± 962.57		13	107.14± 131.10		10	1,526.40± 1,064.53		11	363.58± 656.57		10	1,049.00± 1,080.11		11	502.99± 749.88	
Yes	14	199.36± 133.45		13	166.19± 145.55		13	798.31± 400.64		14	125.23± 144.70		8	1,309.50± 657.32		11	547.81± 1,058.95		13	2,057.31± 2,294.65		13	1,557.49± 2,610.63	
SSL			0.783			0.633			0.251			0.783			0.809			0.549			0.410			0.213
No	12	223.17± 55.45		12	161.98± 111.44		11	1,171.46± 988.31		12	110.50± 136.34		10	1,526.40± 1,064.53		10	391.72± 685.06		9	1,317.67± 1,103.91		10	489.49± 789.03	
Yes	10	235.34± 138.77		10	189.83± 157.19		9	758.33± 358.98		11	127.92± 163.41		6	1,405.17± 714.84		9	654.78± 1,153.65		9	2,140.00± 2,697.21		10	1,737.47± 2,952.34	
BMI, kg/m^2																								
SPL	22	−0.061*	0.787	21	−0.410*	0.065	21	−0.012*	0.958	22	−0.397*	0.067	18	0.142*	0.574	16	−0.242*	0.366	19	−0.230*	0.344	**19**	**−0.487***	**0.034**
SL	21	−0.155*	0.502	20	−0.405*	0.076	20	−0.012*	0.959	21	−0.398*	0.074	17	0.161*	0.537	16	−0.242*	0.366	18	−0.224*	0.371	**18**	**−0.484***	**0.042**
SSL	18	−0.275*	0.270	18	−0.390*	0.110	17	−0.021*	0.937	19	−0.392*	0.097	15	0.131*	0.643	15	−0.216*	0.440	15	−0.292*	0.291	16	−0.489*	0.055
Dimension, mm																								
SPL			–			–			–			–			–			–			–			–
<10	0	–		0	–		0	–		0	–		0	–		0	–		0	–		0	–	
≥10	27	216.48± 106.59		26	161.27± 125.92		25	950.20± 728.67		27	116.52± 135.96		18	1,430.00± 888.91		22	455.70± 864.96		23	1,775.44± 1,859.38		24	1,074.18± 2,021.98	
SL			–			–			–			–			–			–			–			–
<10	0	–		0	–		0	–		0	–		0	–		0	–		0	–		0	–	
≥10	27	216.48± 106.59		26	161.27± 125.92		25	950.20± 728.67		27	116.52± 135.96		18	1,430.00± 888.91		22	455.70± 864.96		23	1,775.44± 1,859.38		24	1,074.18± 2,021.98	

GE Port J Gastroenterol 2024;31:338–350
DOI: 10.1159/000535920

Table 3 (continued)

Variable	Epithelial L-PHA			Epithelial GNA			T CD4+ L-PHA			T CD4+ GNA			T CD8+ L-PHA			T CD8+ GNA			Treg L-PHA			Treg GNA		
	N	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value
SSL			–			–			–			–			–			–			–			-
<10	0	–		0	–		0	–		0	–		0	–		0	–		0	–		0	–	
≥10	22	228.70± 99.51		22	174.64± 131.51		20	985.55± 782.82		23	118.83± 146.67		16	1,480.94± 924.09		19	516.33± 918.90		18	1,728.83± 2,043.52		20	1,113.48± 2,198.53	
Dysplasia																								
SPL			–			–			–			0.312			–			0.548			–			–
No	26	213.19± 107.30		25	161.48± 128.51		24	948.96± 744.32		25	124.14± 138.46		17	1,437.35± 915.70		20	491.86± 900.91		22	1,827.23± 1,886.08		23	1,119.37± 2,054.99	
Yes	1	302.00		1	156.00		1	980.00		2	21.20± 29.98		1	1,305.00		2	94.05± 49.43		1	636.00		1	34.70	
SL			–			–			–			0.312			–			0.548			–			–
No	26	213.19± 107.30		25	161.48± 128.51		24	948.96± 744.32		25	124.14± 138.46		17	1,437.35± 915.70		20	491.86± 900.91		22	1,827.23± 1,886.08		23	1,119.37± 2,054.99	
Yes	1	302.00		1	156.00		1	980.00		2	21.20± 29.98		1	1,305.00		2	94.05± 49.43		1	636.00		1	34.70	
SSL			–			–			–			0.336			–			0.508			–			–
No	21	225.21± 100.58		21	175.53± 134.69		19	985.84± 804.27		21	128.13± 150.24		15	1,492.67± 955.29		17	566.01± 961.70		17	1,793.12± 2,087.57		19	1,170.26± 2,243.66	
Yes	1	302.00		1	156.00		1	980.00		2	21.20± 29.98		1	1,305.00		2	94.05± 49.43		1	636.00		1	34.70	
Synchronous CA																								
SPL			0.699			0.171			0.265			0.096			0.433			0.051			0.398			0.219
No	21	228.11± 111.51		20	178.38± 137.86		19	855.74± 514.56		21	131.39± 150.07		14	1,227.86± 536.31		18	540.11± 939.40		17	1,940.65± 2,026.58		18	1,347.82± 2,277.49	
Yes	7	252.51± 216.53		7	102.01± 40.83		7	1,214.00± 1,104.78		7	68.86± 41.06		5	1,832.00± 1,508.10		4	75.85± 18.89		7	1,228.14± 1,213.32		7	242.51± 279.52	
SL			0.298			0.212			0.469			0.297			0.325			0.051			0.486			0.260
No	21	228.11± 111.51		20	178.38± 137.86		19	855.74± 514.56		21	131.39± 150.07		14	1,227.86± 536.31		18	540.11± 939.40		17	1,940.65± 2,026.58		18	1,347.82± 2,277.49	
Yes	6	175.77± 82.36		6	104.22± 44.57		6	1,249.33± 1,205.89		6	64.48± 43.15		4	2,137.500± 1,540.48		4	75.85± 18.89		6	1,307.33± 1,309.16		6	253.27± 304.61	
SSL			0.753			0.309			0.496			**0.025**			0.390			0.053			0.511			0.350
No	18	231.97± 109.25		18	188.42± 142.03		16	874.44± 552.45		**19**	**134.44± 157.42**		13	1,270.85± 532.51		16	597.91± 984.00		14	1,904.64± 2,237.02		16	1,349.86± 2,411.30	
Yes	4	214.00± 36.47		4	112.65± 22.18		4	1,430.00± 1,423.34		**4**	**44.70± 13.82**		3	2,391.33± 1,781.31		3	81.23± 19.02		4	1,113.50± 1,153.06		4	167.98± 225.08	
Synchronous CA number																								
SPL	25	−0.031*	0.882	24	−0.256*	0.228	**23**	**0.475***	**0.022**	25	−0.274*	0.185	**16**	**0.602***	**0.014**	19	−0.116*	0.637	21	−0.134*	0.562	22	−0.282*	0.204
SL	24	−0.162*	0.449	23	−0.245*	0.260	**22**	**0.477***	**0.025**	24	−0.271*	0.201	**15**	**0.655***	**0.008**	19	−0.116*	0.637	20	−0.121*	0.612	21	−0.273*	0.231
SSL	19	−0.120*	0.625	19	−0.264*	0.274	**17**	**0.493***	**0.044**	20	−0.272*	0.246	**13**	**0.727***	**0.005**	16	−0.118*	0.664	15	−0.162*	0.564	17	−0.247*	0.339
Location																								
SPL			0.538			0.739			0.068			0.382			0.022			0.390			0.726			0.928
Right colon	19	254.63± 165.58		18	155.18± 124.04		18	749.67± 379.37		19	91.29± 120.16		14	1,068.14± 430.38		15	632.67± 1,007.77		18	1,574.50± 2,021.34		18	1,072.38± 2,288.92	
Transverse colon	7	197.14± 46.55		7	183.21± 145.79		7	1,476.29± 1,128.23		7	164.36± 163.98		4	2,238.75± 1,459.49		5	96.64± 44.70		5	2,078.00± 1,150.33		5	1,131.48± 1,086.42	
Left colon	2	170.00± 16.97		2	106.10± 39.46		1	929.00		2	178.05± 155.49		1	2,396.00		2	26.00± 36.77		1	2,857.00		2	499.05± 609.46	

Table 3 (continued)

Variable		Epithelial L-PHA			Epithelial GNA			T CD4+ L-PHA			T CD4+ GNA			T CD8+ L-PHA			T CD8+ GNA			Treg L-PHA			Treg GNA		
		N	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value
SL				0.666			0.755			0.071			0.401			0.033			0.390			0.764			0.922
	Right colon	18	229.17± 126.43		17	158.72± 126.92		17	734.82± 385.62		18	91.08± 123.64		13	1,106.85± 421.83		15	632.67± 1,007.77		17	1,622.82± 2,071.80		17	1,124.99± 2,348.12	
	Transverse colon	7	197.14± 46.55		7	183.21± 145.79		7	1,476.29± 1,128.23		7	164.36± 163.98		4	2,238.75± 1,459.49		5	96.64± 44.70		5	2,078.00± 1,150.33		5	1,131.48± 1,086.42	
	Left colon	2	170.00± 16.97		2	106.10± 39.46		1	929.00		2	178.05± 155.49		1	2,396.00		2	26.00± 36.77		1	2,857.00		2	499.05± 609.46	
SSL				0.537			0.707			0.101			0.479			0.058			0.387			0.825			0.892
	Right colon	14	245.53± 119.87		14	173.91± 133.54		13	730.00± 350.15		15	90.97± 134.94		11	1,122.18± 432.27		13	719.09± 1,060.26		13	1,571.92± 2,312.86		14	1,259.94± 2,579.78	
	Transverse colon	6	209.00± 39.14		6	199.18± 152.85		6	1,548.67± 1,217.98		6	168.75± 179.17		4	2,238.75± 1,459.49		4	102.50± 49.35		4	1,956.75± 1,290.87		4	908.10± 1,114.04	
	Left colon	2	170.00± 16.97		2	106.10± 39.46		1	929.00		2	178.05± 155.29		1	2,396.00		2	26.00± 36.77		1	2,857.00		2	499.05± 609.46	
Adenocarcinoma SPL																									
	No	27	216.48± 106.59		26	161.27± 125.92		25	959.20		27	116.52± 135.96		18	1,430.00± 888.91		22	455.70± 864.96		23	1,775.44± 1,859.38		24	1,074.18± 2,021.98	
	Yes	1	713.00		1	95.10		1	1,002.00		1	95.10		1	565.00		0	–		1	753.00		1	178.00	

BMI, body mass index; *CA*, conventional adenoma; GNA, *Glanthus nivalis agglutinin*; L-PHA, *Phaseolus vulgaris leucoagglutinin*; SL, serrated lesion; SPL, serrated pathway lesion; SSL, sessile serrated lesion; *r*, correlation coefficient; Treg, regulatory T cell; FC, flow cytometry. *Correlation coefficient.

epithelial component is also correlated with smoking and polyp dimensions ≥10 mm. Previously, our group showed that these types of glycans present an aberrant expression in CRC and are direct immune modulators in the tumor microenvironment, allowing immune evasion [14]. Thus, these clinical variables may intervene as risk factors for the progression to malignancy in the serrated pathway by enabling identification of immunological escape in these lesions. In fact, age is a known risk factor for the CRC development and progression, with immunosenescence potentially playing an important role in the immune escape suggested in our results [1, 17]. Smoking is a studied and validated risk factor for SL development and progression to CRC [17, 18]. This evidence is in line with our results, emphasizing smoking cessation as a method of preventing progression in the serrated pathway. Additionally, BMI has been described in the literature as a possible risk factor for CRC progression [19]. However, it is not fully understood whether it impacts the adenoma-carcinoma pathway or the serrated pathway [19]. Our findings suggest that BMI may contribute to progression to malignancy in the serrated pathway by upregulating β1,6-GlcNAc branched *N*-glycans in epithelial cells. Thereby, weight loss should be encouraged in individuals with SPL in an attempt to prevent progression to malignancy. Regarding SL dimensions, our results suggest that polyps with dimensions ≥10 mm have greater risk of progression to malignancy, by overexpression of β1,6-GlcNAc branched *N*-glycans in epithelial cells. This result is in line with the European guideline for post-polypectomy colonoscopy follow-up, which defined a cut-off of 10 mm to perform a shorter interval follow-up [20].

Regarding the immune compartment, it was observed a higher β1,6-GlcNAc branched *N*-glycans expression with the increasing number of synchronous CA in patients with SPL. Previously, our group had shown that an increasing β1,6-GlcNAc branched *N*-glycans expression in T cells, by GlcNAc supplementation, controls T-cell immune response in inflammatory bowel disease, by increasing its threshold of activation [16]. Thus, T-cell β1,6-GlcNAc branched *N*-glycosylation seems to create an immunosuppressive environment disabling T-cell activation and function in SPL, promoting their growth. This immunosuppression promotes the development of more synchronous CA. Therefore, a higher synchronous CA number seems to be a risk factor for progression to malignancy in the serrated pathway. Synchronous CA number has not been described in the literature as a risk factor for development or progression to malignancy in the serrated pathway yet. Thereby, the anatomopathological identification of the *N*-glycosylation pattern in the presence of a high synchronous CA number can select patients that would

GE Port J Gastroenterol 2024;31:338–350
DOI: 10.1159/000535920

Table 4. N-glycosylation profile results obtained by MGAT5 gene RT-qPCR and histochemistry

Variable	*MGAT5* gene RT-qPCR			Epithelial L-PHA			Epithelial GNA			Stromal L-PHA			Stromal GNA		
	N	mean/*r**	*p* value	*n*	mean/*r**	*p* value	*n*	mean/*r**	*p* value	*n*	mean/*r**	*p* value	*n*	mean/*r**	*p* value
Gender															
SPL			0.124			0.338			0.387			0.628			0.943
Male	16	$3.83 \times 10^{-4} \pm 5.97 \times 10^{-4}$		6	0.92±0.92		6	1.17±1.13		6	0.83±0.52		6	1.54±1.14	
Female	17	$1.35 \times 10^{-4} \pm 1.31 \times 10^{-4}$		6	1.42±0.80		6	0.67±0.75		6	1.00±0.63		6	1.58±0.80	
SL			0.240			0.102			0.677			0.601			0.172
Male	14	$1.37 \times 10^{-4} \pm 1.35 \times 10^{-4}$		4	0.88±0.85		4	0.50±0.41		4	0.75±0.65		4	0.81±0.24	
Female	16	$2.39 \times 10^{-4} \pm 2.88 \times 10^{-4}$		5	1.70±0.45		5	0.70±0.84		5	1.00±0.71		5	1.50±0.87	
SSL			0.273			0.143			0.725			0.553			0.205
Male	14	$1.43 \times 10^{-4} \pm 1.37 \times 10^{-4}$		3	0.83±1.04		3	0.50±0.50		3	0.67±0.76		3	0.75±0.25	
Female	15	$2.39 \times 10^{-4} \pm 2.88 \times 10^{-4}$		5	1.70±0.45		5	0.70±0.84		5	1.00±0.71		5	1.50±0.87	
Age, years															
SPL	33	0.139*	0.440	12	−0.361*	0.263	12	−0.261*	0.650	12	0.146*	0.412	12	0.040*	0.901
SL	30	0.017*	0.930	9	−0.454*	0.219	9	−0.329*	0.310	9	−0.382*	0.387	9	−0.479*	0.192
SSL	29	0.016*	0.933	8	−0.451*	0.262	8	−0.335*	0.354	8	−0.379*	0.418	8	−0.476*	0.233
Smoking															
SPL			0.878			0.265			0.106			0.780			**0.010**
No	15	$2.68 \times 10^{-4} \pm 5.95 \times 10^{-4}$		3	0.67±1.15		3	1.84±1.26		3	1.00±0.00		**3**	**2.67±0.58**	
Yes	18	$2.45 \times 10^{-4} \pm 2.62 \times 10^{-4}$		9	1.33±0.75		9	0.61±0.65		9	0.89±0.65		**9**	**1.19±0.73**	
SL			**0.038**			–			–	–		–			–
No	**12**	$\mathbf{9.49 \times 10^{-5} \pm 9.82 \times 10^{-5}}$		0	–		0	–		0	–		0	–	
Yes	**18**	$\mathbf{2.50 \times 10^{-4} \pm 2.62 \times 10^{-4}}$		9	1.33±0.75		9	0.61±0.65		9	0.89±0.65		9	1.19±0.73	
SSL			0.053			–			–			–			–
No	11	$9.99 \times 10^{-5} \pm 1.01 \times 10^{-4}$		0	–		0	–		0	–		0	–	
Yes	18	$2.45 \times 10^{-4} \pm 2.62 \times 10^{-4}$		8	1.38±0.79		8	0.63±0.69		8	0.88±0.69		8	1.22±0.77	
BMI, kg/m^2															
SPL	**22**	**0.432***	**0.045**	8	0.458*	0.254	8	−0.115*	0.516	8	0.271*	0.786	8	0.371*	0.445
SL	19	0.428*	0.068	5	0.566*	0.320	5	−0.115*	0.178	5	−0.711*	0.853	5	−0.032*	0.959
SSL	**18**	**0.496***	**0.036**	4	0.698*	0.302	4	−0.154*	0.236	4	−0.764*	0.846	4	−0.005*	0.995
Dimension, mm															
SPL			0.427			–			–			–			–
<10	3	$4.40 \times 10^{-5} \pm 3.96 \times 10^{-6}$		0			0	–		0	–		0	–	
≥10	29	$2.52 \times 10^{-4} \pm 4.40 \times 10^{-4}$		12	1.17±0.86		12	0.92±0.95		12	0.92±0.56		12	1.56±0.94	
SL			**0.001**			–			–			–			–
<10	**3**	$\mathbf{4.40 \times 10^{-5} \pm 3.96 \times 10^{-6}}$		0	–		0	–		0	–		0	–	
≥10	**26**	$\mathbf{1.70 \times 10^{-4} \pm 1.68 \times 10^{-4}}$		9	1.33±0.75		9	0.61±0.65		9	0.89±0.65		9	1.19±0.73	

GE Port J Gastroenterol 2024;31:338–350
DOI: 10.1159/000535920

Table 4 (continued)

Variable	*MGAT5* gene RT-qPCR			Epithelial L-PHA			Epithelial GNA			Stromal L-PHA			Stromal GNA		
	N	mean/*r**	*p* value	*n*	mean/*r**	*p* value	*n*	mean/*r**	*p* value	*n*	mean/*r**	*p* value	*n*	mean/*r**	*p* value
SSL			0.316			–			–			–			–
<10	2	$4.61 \times 10^{-5} \pm 2.26 \times 10^{-6}$		0	–		0	–		0	–		0	–	
≥10	26	$1.70 \times 10^{-4} \pm 1.68 \times 10^{-4}$		8	1.38±0.79		8	0.63±0.69		8	0.88±0.69		8	1.22±0.77	
Dysplasia															
SPL			0.084			0.755			0.241			1			**0.028**
No	24	$1.75 \times 10^{-4} \pm 2.38 \times 10^{-4}$		6	1.08±0.80		6	0.58±0.38		6	0.92±0.80		**6**	**0.96±0.33**	
Yes	9	$4.70 \times 10^{-4} \pm 7.28 \times 10^{-4}$		6	1.25±0.99		6	1.25±1.26		6	0.92±0.20		**6**	**2.17±0.98**	
SL			0.637			0.170			0.913			0.870			0.800
No	24	$1.75 \times 10^{-4} \pm 2.38 \times 10^{-4}$		6	1.08±0.80		6	0.58±0.38		6	0.92±0.80		6	0.96±0.33	
Yes	6	$2.24 \times 10^{-4} \pm 1.51 \times 10^{-4}$		3	1.83±0.29		3	0.67±1.15		3	0.83±0.29		3	1.67±1.15	
SSL			0.683			0.229									
No	23	$1.81 \times 10^{-4} \pm 2.42 \times 10^{-4}$		5	1.10±0.89		5	0.60±0.42		5	0.90±0.89		5	0.95±0.37	
Yes	6	$2.24 \times 10^{-4} \pm 1.51 \times 10^{-4}$		3	1.83±0.29		3	0.68±1.15		3	0.83±0.29		3	1.67±1.15	
Synchronous CA															
SPL			0.646			0.265			0.406			0.780			**0.010**
No	23	$2.79 \times 10^{-4} \pm 5.15 \times 10^{-4}$		9	1.33±0.87		9	1.06±1.04		9	0.94±0.53		**9**	**1.83±0.94**	
Yes	10	$2.02 \times 10^{-4} \pm 1.60 \times 10^{-4}$		3	0.67±0.76		3	0.50±0.50		3	0.83±0.76		**3**	**0.75±0.25**	
SL			0.778			0.208			0.743			0.870			0.214
No	20	$1.76 \times 10^{-4} \pm 2.51 \times 10^{-4}$		6	1.67±0.52		6	0.67±0.75		6	0.92±0.66		6	1.42±0.80	
Yes	10	$2.01 \times 10^{-4} \pm 1.60 \times 10^{-4}$		3	0.67±0.76		3	0.50±0.50		3	0.83±0.76		3	0.75±0.25	
SSL			0.845			**0.035**			0.725			0.907			0.205
No	19	$1.84 \times 10^{-4} \pm 2.56 \times 10^{-4}$		**5**	**1.80±0.45**		5	0.70±0.84		5	0.90±0.74		5	1.50±0.87	
Yes	10	$2.01 \times 10^{-4} \pm 1.60 \times 10^{-4}$		**3**	**0.68±0.76**		3	0.50±0.50		3	0.83±0.76		3	0.75±0.25	
Synchronous CA number															
SPL	27	−0.074*	0.713	10	−0.308*	0.387	10	−0.551*	0.621	10	−0.179*	0.099	10	−0.501*	0.140
SL	25	0.037*	0.861	8	−0.607*	0.110	8	−0.569*	0.770	8	−0.124*	0.141	8	−0.481*	0.227
SSL	25	0.037*	0.861	7	−0.658*	0.108	7	−0.583*	0.742	7	−0.154*	0.170	7	**−0.522***	0.230
Location															
SPL	33		0.332	12		0.567	**12**		**0.009**	12		0.855	**12**		**0.039**
Right colon	20	$1.90 \times 10^{-4} \pm 2.29 \times 10^{-4}$		5	1.50±0.87		**5**	**0.80±0.84**		5	1.00±0.35		**5**	**1.35±0.93**	
Transverse colon	7	$2.35 \times 10^{-4} \pm 2.42 \times 10^{-4}$		5	0.90±0.74		**5**	**0.40±0.22**		5	0.80±0.84		**5**	**1.20±0.57**	
Left colon	6	$4.96 \times 10^{-4} \pm 9.31 \times 10^{-4}$		2	1.00±1.41		**2**	**2.50±0.71**		2	1.00±0.00		**2**	**3.00±0.00**	

Table 4 (continued)

Variable	*MGAT5* gene RT-qPCR			Epithelial L-PHA			Epithelial GNA			Stromal L-PHA			Stromal GNA		
	N	mean/*r**	*p* value	*n*	mean/*r**	*p* value	*n*	mean/*r**	*p* value	*n*	mean/*r**	*p* value	*n*	mean/*r**	*p* value
SL			0.362			–			–			–			–
Right colon	20	$1.90 \times 10^{-4} \pm 2.29 \times 10^{-4}$		5	1.50±0.87		5	0.80±0.84		5	1.00±0.35		5	1.35±0.93	
Transverse colon	6	$2.55 \times 10^{-4} \pm 2.59 \times 10^{-4}$		4	1.13±0.63		4	0.38±0.25		4	0.75±0.96		4	1.00±0.41	
Left colon	4	$4.95 \times 10^{-5} \pm 4.61 \times 10^{-6}$		0	–		0	–		0	–		0	–	
SSL			0.362			–			–			–			–
Right colon	19	$1.98 \times 10^{-4} \pm 2.32 \times 10^{-4}$		5	1.50±0.87		5	0.80±0.84		5	1.00±0.35		5	1.35±0.93	
Transverse colon	6	$2.55 \times 10^{-4} \pm 2.59 \times 10^{-4}$		3	1.17±0.76		3	0.33±0.29		3	0.67±1.15		3	1.00±0.50	
Left colon	4	$4.50 \times 10^{-5} \pm 4.61 \times 10^{-5}$		0	–		0	–		0	–		0	–	
Adenocarcinoma															
SPL			0.388			0.265			0.106			0.780			**0.010**
No	30	$1.85 \times 10^{-4} \pm 2.22 \times 10^{-4}$		9	1.33±0.75		9	0.61±0.65		9	0.89±0.65		**9**	**1.19±0.73**	
Yes	3	$9.63 \times 10^{-4} \pm 1.23 \times 10^{-3}$		3	0.67±1.15		3	1.83±1.26		3	1.00±0.00		**3**	**2.67±0.58**	

BMI, body mass index; CA, conventional adenoma; GNA, *Glanthus nivalis agglutinin*; L-PHA, *Phaseolus vulgaris leucoagglutinin*; SL, serrated lesion; SPL, serrated pathway lesion; SSL, sessile serrated lesion; *r*, correlation coefficient; RT-PCR, reverse transcriptase-quantitative polymerase chain reaction. *Correlation coefficient.

benefit from a shorter interval of follow-up, to reduce interval cancers. However, we did not distinguish the dysplasia type of CA (high-grade vs. low-grade dysplasia), which may limit these conclusions.

According to our data, smoking and synchronous CA presence seem to be risk factors for progression to CRC in the serrated pathway, since they are correlated with high-mannose *N*-glycans downregulation on T cells, suggesting decreased immune function in SPL. Furthermore, it seems that the lower immune system activation by downregulation of high-mannose *N*-glycans enables the synchronous CA development. In fact, Li et al. [21] verified that the synchronous CA presence in patients with SL confers an increased risk of developing CRC. Regarding this, our results are in accordance with the literature, emphasizing *N*-glycosylation as a new potential biomarker for malignancy progression in serrated pathway. Contrariwise, we also showed an increased stromal high-mannose *N*-glycans expression in the presence of dysplasia and adenocarcinoma with concomitant SSL-D and in the left colon lesions. These findings suggest that the immune system is more active both in the presence of dysplasia and adenocarcinoma of the serrated pathway, contradicting what we expected. We predicted a reduced high-mannose *N*-glycans in immune cells related with T cells inability to recognize neoplastic lesions. Thereby, we need further investigations with a larger sample size to clarify these results. Regarding SPL location, left colon lesions appear to have a more active immunological profile, by having a higher high-mannose *N*-glycans expression, suggesting that this location is less likely to progress to CRC. In fact, SPL is more frequent in the right colon [22]. These results have a follow-up impact, considering the low potential for progression of a left colon lesion. Therefore, the *N*-glycosylation profile in colonic immune compartment could be used to guide clinicians on follow-up decision.

As previously mentioned, we found an association between the β1,6-GlcNAc branched *N*-glycans in epithelial component and a high BMI. An increased BMI was also associated with a reduced high-mannose *N*-glycans expression on Tregs. These results suggest that Tregs might have an increased immunosuppressive capacity in the presence of elevated BMI, creating an immunosuppressive microenvironment, which contributes to progression to malignancy. Thus, as emphasized earlier, patients with higher BMI may be considered for a closer surveillance of SPL.

Regarding gender, a different *N*-glycosylation pattern was observed in different immune system cells. In fact, β1,6-GlcNAc *N*-glycosylation in different genders has

never been studied before. Our results showed a higher high-mannose *N*-glycosylation expression in $CD4^+$ T cells in females and in $CD8^+$ T cells in males in all serrated pathway, highlighting that men and women have higher activation of different T cells. Both $CD8^+$ and $CD4^+$ T cells play a role in tumor eradication by direct action ($CD8^+$ T cells) and cytokine release ($CD4^+$ T cells) [12]. Despite this, $CD8^+$ T cells infiltration in tumor microenvironment is correlated with a better prognosis in CRC, suggesting that men have lower progression to cancer in serrated pathway [23]. By opposition, Tregs in females have a higher β1,6-GlcNAc branched *N*-glycans expression creating an immunosuppressive environment that allows the progression to malignancy. Regarding this, our results suggest a different *N*-glycosylation pattern of immune system cells occurring in both genders, which demands further investigation.

To conclude, biomarkers are an essential tool in current medical practice, playing an important role on understanding and identifying several diseases. Our study showed that *N*-glycosylation could be a potential biomarker of tumor progression in the serrated pathway. This study set the ground for the potential inclusion of β1,6-GlcNAc branched *N*-glycans and high-mannose *N*-glycans in the SPL anatomopathological analysis to select those patients who need shorter intervals of follow-up to reduce the interval cancers incidence. Furthermore, according to *N*-glycosylation profile, we identified smoking, aging, elevated BMI, SL dimensions ≥10 mm, the presence and number of synchronous CA as risk factors to progression to malignancy. These associations allow directed clinical interventions based on risk factors to reduce the progression to malignancy. Taken together, *N*-glycosylation profile seems to be one key to solve this puzzling pathway with large impact in clinical and molecular research. Despite our results, this study has several limitations, namely, the small sample size, the high missing data value, and the lack of similar articles that prevent us to draw more conclusions.

Statement of Ethics

This study protocol was reviewed and approved by Departamento de Ensino, Formação e Investigação (DEFI), and ethical committee of Centro Hospitalar Universitário de Santo António, number 2021.306 (252-DEFI/260-CE). Informed consent was not required, decided by ethical committee of Centro Hospitalar Universitário de Santo António.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This article had the funding support of Liga Portuguesa Contra o Cancro (LPCC) and MultiCare (Grant/Award No. C 3035006C17). This work was also funded by Raquel Seruca Porto Comprehensive Cancer Centre (P.CCC). Catarina M. Azevedo thanks Fundação para a Ciência e Tecnologia (FCT) for a fellowship (2021.07357.BD).

Author Contributions

Henrique Fernandes-Mendes: conceptualization; methodology; investigation; formal analysis; and writing – original draft; Catarina M. Azevedo: conceptualization; methodology; investigation; and writing – review and editing. Mónica Garrido: conceptualization and writing – review and editing. Carolina Lemos: methodology and formal analysis. Isabel Pedroto: supervision. Salomé S. Pinho: writing – review and editing and supervision. Ricardo Marcos-Pinto and Ângela Fernandes: conceptualization; methodology; investigation; formal analysis; writing – review and editing; and supervision. All authors approved the final version of the manuscript.

Data Availability Statement

This article was based on a final master's thesis of medical training, which will be published on Repositório da Universidade do Porto on June 28, 2023.

References

1 Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. Lancet. 2019;394(10207):1467–80.
2 Roquette R, Painho M, Nunes B. Geographical patterns of the incidence and mortality of colorectal cancer in mainland Portugal municipalities (2007–2011). BMC Cancer. 2019;19(1):512.
3 Crockett SD, Nagtegaal ID. Terminology, molecular features, epidemiology, and management of serrated colorectal neoplasia. Gastroenterology. 2019;157(4):949–66.e4.
4 Kim JH, Kang GH. Evolving pathologic concepts of serrated lesions of the colorectum. J Pathol Transl Med. 2020;54(4):276–89.
5 Bell PD, Anderson JC, Srivastava A. The frontiers of serrated polyps. Am J Surg Pathol. 2022;46(1):E64–70.
6 Okamoto K, Kitamura S, Kimura T, Nakagawa T, Sogabe M, Miyamoto H, et al. Clinicopathological characteristics of serrated polyps as precursors to colorectal cancer: current status and management. J Gastroenterol Hepatol. 2017;32(2):358–67.
7 Rashtak S, Rego R, Sweetser SR, Sinicrope FA. Sessile serrated polyps and colon cancer prevention. Cancer Prev Res. 2017;10(5):270–8.

8 Acosta-Gonzalez G, Ouseph M, Lombardo K, Lu S, Glickman J, Resnick MB. Immune environment in serrated lesions of the colon: intraepithelial lymphocyte density, PD-1, and PD-L1 expression correlate with serrated neoplasia pathway progression. Hum Pathol. 2019;83:115–23.
9 Murakami T, Sakamoto N, Nagahara A. Clinicopathological features, diagnosis, and treatment of sessile serrated adenoma/polyp with dysplasia/carcinoma. J Gastroenterol Hepatol. 2019;34(10):1685–95.
10 O'Connell BM, Crockett SD. The clinical impact of serrated colorectal polyps. Clin Epidemiol. 2017;9:113–25.
11 Pinho SS, Reis CA. Glycosylation in cancer: mechanisms and clinical implications. Nat Rev Cancer. 2015;15(9):540–55.
12 Fernandes Â, Azevedo CM, Silva MC, Faria G, Dantas CS, Vicente MM, et al. Glycans as shapers of tumour microenvironment: a sweet driver of T-cell-mediated anti-tumour immune response. Immunology. 2023;168(2):217–32.
13 Pereira MS, Alves I, Vicente M, Campar A, Silva MC, Padrão NA, et al. Glycans as key checkpoints of T cell activity and function. Front Immunol. 2018;9:2754.
14 Silva MC, Fernandes Â, Oliveira M, Resende C, Correia A, de-Freitas-Junior JC, et al. Glycans as immune checkpoints: removal of branched N-glycans enhances immune recognition preventing cancer progression. Cancer Immunol Res. 2020;8(11):1407–25.
15 Demetriou M, Granovsky M, Quaggin S, Dennis JW. Negative regulation of T-cell activation and autoimmunity by Mgat5 N-glycosylation. Nature. 2001;409(6821):733–9.
16 Dias AM, Correia A, Pereira MS, Almeida CR, Alves I, Pinto V, et al. Metabolic control of T cell immune response through glycans in inflammatory bowel disease. Proc Natl Acad Sci U S A. 2018;115(20):E4651–60.
17 Bouwens MWE, Winkens B, Rondagh EJA, Driessen AL, Riedl RG, Masclee AAM, et al. Simple clinical risk score identifies patients with serrated polyps in routine practice. Cancer Prev Res. 2013;6(8):855–63.
18 He X, Wu K, Ogino S, Giovannucci EL, Chan AT, Song M. Association between risk factors for colorectal cancer and risk of serrated polyps and conventional adenomas. Gastroenterology. 2018;155(2):355–73.e18.
19 Bailie L, Loughrey MB, Coleman HG. Lifestyle risk factors for serrated colorectal polyps: a systematic review and meta-analysis. Gastroenterology. 2017;152(1):92–104.
20 Hassan C, Antonelli G, Dumonceau JM, Regula J, Bretthauer M, Chaussade S, et al. Post-polypectomy colonoscopy surveillance: European society of gastrointestinal endoscopy (ESGE) guideline – update 2020. Endoscopy. 2020;52(8):687–700.
21 Li D, Doherty AR, Raju M, Liu L, Lei NY, Amsden LB, et al. Risk stratification for colorectal cancer in individuals with subtypes of serrated polyps. Gut. 2021:gutjnl-2021-324301.
22 Cassese G, Amendola A, Maione F, Giglio MC, Pagano G, Milone M, et al. Serrated lesions of the colon-rectum: a focus on new diagnostic tools and current management. Gastroenterol Res Pract. 2019;2019:9179718.
23 Koch M, Beckhove P, Op Den Winkel J, Autenrieth D, Wagner P, Nummer D, et al. Tumor infiltrating T lymphocytes in colorectal cancer: tumor-selective activation and cytotoxic activity in situ. Ann Surg. 2006;244(6):986–92; discussion 992–3.
24 Macarie M, Bățagă S, Mocan S, Pantea M, Opaschi R, Voidazan S, et al. Correlation of metabolic risk factors with sessile serrated lesions. J Gastrointest Liver Dis. 2020;29(2):175–9.
25 Anwar S, Cock C, Young J, Young GP, Meng R, Simpson K, et al. Features associated with high-risk sessile serrated polyps at index and follow-up colonoscopy. J Gastroenterol Hepatol. 2021;36:1620–6.
26 Symonds E, Anwar S, Young G, Meng R, Coats M, Simpson K, et al. Sessile serrated polyps with synchronous conventional adenomas increase risk of future advanced neoplasia. Dig Dis Sci. 2019;64(6):1680–5.