OPTIMIZING STOOL GLUTEN IMMUNOGENIC PEPTIDE TESTS FOR THE MONITORING OF ADHERENCE TO THE GLUTEN-FREE DIET.

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Background: The only accepted treatment for celiac disease (CeD) is lifelong strict adherence to a gluten-free diet (GFD). Current tools for assessing GFD adherence have limitations and new quantitative and qualitative tests that detect gluten immunogenic peptides (GIP) in stool and urine have recently been developed. However, evidence regarding the best strategy for their use in clinical practice is lacking.
Aim: We investigated the optimal frequency of GIP measurement in patients with CeD on a GFD. **Methods:** A prospective observational study was conducted on patients with non-complicated CeD while performing their usual GFD, for more than 2 years. Patients were asked to collect stool samples once or twice during four consecutive seven-day intervals. First stool collection was on Friday/Saturday (F/S) and the second on Tuesday (T) for each interval. GIP in stool was measured by ELISA (limit of detection: >0.156 µg/g). Based on previous studies investigating the threshold of gluten contamination needed for mucosal damage and a conversion formula, we estimated that an average stool GIP ≥0.32 µg/g/7 days represents gluten exposure >50 mg/day (50% association with mucosal damage), while ≥0.64 µg/g represents an average daily gluten intake >100 mg/day (100% association with mucosal damage). **Results**: We enrolled 22 patients who collected 176 stool samples and GIP was found in 83 (47.2%). Only four (18.2%) patients had no GIP during the four intervals, and four others had ≤ two out of eight samples positive (super-adherents/rare indiscretions). Six other patients (27.2%) had GIP in 6-8 out of 8 positive samples (highly frequent indiscretions). Finally, eight patients had positive GIP in 3 to 5 out of eight samples collected. There was no difference in the percent of GIP positive stools comparing single weekly sampling obtained either F/S or T (47.7% vs. 46.6%, respectively). Nineteen out of 22 (86.4%) patients had GIP ≥0.32 µ/g in more than 37.5% of samples. Furthermore, 7/22 (31.8%) had GIP ≥0.64 µg/g/7 day interval in more than 50% of samples. Compared to single 7-day interval stool collection, the strategy of collecting two stool samples per interval detected higher transgressions at both, ≥0.32 and ≥0.64 µg/g levels (p=0.07 and p<0.01, respectively). **Conclusions**: Our study explored two strategies using different frequency of stool GIP testing to assess adherence to the GFD. Stool sampling twice weekly, over a period of 4 weeks, detects a higher frequency of dietary transgressions than once a week. According to our estimation with twice weekly GIP monitoring, a significant proportion of patients is frequently exposed to risky amounts of gluten.

Table 1

	Overall population	**≥0.32 µg/g** of GIP in stool **once/interval**	**≥0.32µg/g** of GIP in stool **twice/interval**	**≥0.65 µg/g** of GIP in stool **once/interval**	**≥0.65 µg/g** of GIP in stool **twice/interval**
N of patients. (%)	22 (100)	11 (50.0)	17 (77.4)*	7 (31.8)	16(72.7)**
Age. Median (25% IQR)	37 (31-46)	37 (27-42)	37 (27-42)	37 (34-42)	38 (29-44)
Female gender. (%)	21 (95.4)	10 (90.9)	16 (94.1)	6 (85.7)	11 (91.7)
Average GIP excretion/ interval. Median (25% IQR). µg/g	0.76 (0.12-6.86)	1.04 (0.45-2.75)	3.86 (1.11-17.11)**	2.56 (1.04-2.87)	6.22 (0.87-30.95.)**
Years from CeD diagnosis. Median (25% IQR)	10 (6-14)	13 (10-19)	12 (7-17)	15 (11-34)	11 (6-14)

Table 1: Demographic data and average stool GIP excretion/seven day/four consecutive intervals in patients with stool GIP concentrations ≥0.32 µg/g or ≥0.65 µg/g as assessed in the overall population, once or twice/seven day interval. 25% IQR: 25% interquartile range. Comparison of GIP stool excretion (≥32 µg/g and ≥0.64 µg/g of stool) determined once/seven day interval vs. twice/seven day interval: * p=0.07; ** p<0.01.

Table 2: Stool GIP concentrations for each sampling (µg/g of stool) on Friday/Saturday (F/S) and Tuesday (T), percentage of positive samples and average stool GIP excretion/patient for the eight samples.

Patient	1st 7-day interval (GIP µg/g of stool)		2nd 7-day interval (GIP µg/g of stool)		3rd 7-day interval (GIP µg/g of stool)		4th 7-day interval (GIP µg/g of stool)		% of +ve samples	Average GIP concentration /8 samples (µg/g)
	F/S	T	F/S	T	F/S	T	F/S	T		
1	0.6	0.34	1.05	0.82	0	0	0	0	50	0.35
2	0	0	0.59	0.48	0	0	0	0	25	0.13
3	0.94	0.69	1.5	0.39	1.31	0	0.41	1.9	87.5	0.89
4	0	0	0.34	0.32	0	0	0	0	25	0.08
5	0.97	0.57	0.39	0.43	5.1	4.67	5.1	6.24	100	2.93
6	0	0	1.27	0.61	1.9	0	0	0	37.5	0.47
7	0	0	0	0	0	0	0	0	0	0.00
8	0	0	0	0	0	0	0	0	0	0.00
9	1.1	1.05	0	0	0.4	0.34	0.71	0.53	75	0.52
10	2.37	0.87	2.75	2.75	2.81	2.49	0.9	1.15	100	2.01
11	0	0	0	0	0	0	0	0	0	0.00
12	0.32	0.47	0	0	0	0	0	0	25	0.10
13	0	0	0	0	0	0	0	0	0	0.00
14	7.4	4.92	0.48	0.74	1.16	0.94	1.95	1.28	100	2.36
15	0	0.46	0	0	0	0.67	0	0	25	0.14
16	1.31	0	0.85	0	6.85	0	2.46	0	50	1.43
17	0	1.05	0	0.91	0	2.39	0	2.38	50	0.84
18	0.5	0.47	0	0	0.43	0	0.69	0	50	0.26
19	0.64	0.46	0	0	1.14	0.32	0	0.56	62.5	0.39
20	0.46	0	2.69	2.2	0.87	0.43	6.23	0.71	87.5	1.70
21	0	0	0	0	0.42	0	0	0.35	25	0.10
22	0.66	0.92	0.62	0	0	0.33	0	1.22	62.5	0.47

Table 3

Categorization	**GIP+ve/n of samples**	**N of patients in each category** (%)	**Median GIP in stool/interval** µg/g (Range)	**Median GIP in stool/interval/ group** µg/g (Range)
Super-adherents	0/8	4 (18.2)	0.0	0.0 (0.0-0.67)
Rare indiscretions	<2/8	5 (22.7)	0.0 (0.0-0.67)	
Frequent indiscretions	3-5/8	7 (31.8)	0.0 (0.0-6.85)	
Very frequent indiscretions	6-7/8	3 (13.6)	0.69 (0.0-6.23)	0.96 (0.0-7.40)
Permanent indiscretions	8/8	3 (13.6)	1.52 (0.39-7.40)	

Table 3: Categorization of 22 CD treated patients according to the degree of adherence to the GFD as estimated by the frequencies of excretion of GIP in stool (GIP +ve/n of samples collected and number of samples +ve in the overall study) assessed twice/period during four consecutive seven-day intervals and averageconcentration /period or median/period, (µg/g). Total number of stool samples collected and analyzed= 8