

University of Groningen

The web around patients with neuroendocrine tumors

Bouma, Grytsje

DOI:
[10.33612/diss.98868349](https://doi.org/10.33612/diss.98868349)

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2019

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):
Bouma, G. (2019). *The web around patients with neuroendocrine tumors: novel ways to inform, support and treat*. Rijksuniversiteit Groningen. <https://doi.org/10.33612/diss.98868349>

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Niacin (vitamin B3) supplementation in patients with serotonin-producing neuroendocrine tumor

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Abstract

Background/Aims

Tryptophan is the precursor of serotonin and niacin (vitamin B3). The latter is critical for normal cellular metabolism. Tryptophan and niacin can be deficient in patients with serotonin-producing neuroendocrine tumors (NETs). Niacin deficiency may lead to severe symptoms including pellagra. In patients with serotonin-producing NET, data on niacin status are scarce and niacin supplementation hardly receives attention. We aimed to assess the niacin status before and after supplementation in these patients.

Methods

We identified serotonin-producing NET patients who had received oral niacin supplementation (mean dose 144 mg daily) for tryptophan deficiency and/or pellagra-associated symptoms. Presupplementation plasma tryptophan levels and niacin status based on the urinary niacin metabolite N1-methylnicotinamide (N1-MN) before (n = 42) and after the start of the supplementation (in 34 paired samples) were assessed. Reference values for urinary N1-MN levels were established in 133 healthy individuals.

Results

The mean presupplementation plasma tryptophan level was 31.8 ± 9.7 $\mu\text{mol/l}$ (reference value 40.0-70.0). Presupplementation urinary N1-MN levels were lower in patients (median 17.9 $\mu\text{mol}/24$ h, range 2.6-70.3) compared to healthy controls (median 43.7 $\mu\text{mol}/24$ h, range 9.5-169.3, $p < 0.0001$) and below normal in 45% of the patients. Niacin supplementation increased urinary N1-MN levels to high normal levels (median 55.5 $\mu\text{mol}/24$ h, range 7.4-489.0) in 86% of the niacin-deficient patients.

Conclusion

In serotonin-producing NET patients, niacin deficiency is prevalent. Therefore, urinary N1-MN deserves to be included in their standard biochemical evaluation. Niacin supplementation normalizes the niacin status in most niacin-deficient serotonin-producing NET patients. A prospective study is warranted.

Keywords: neuroendocrine tumor, serotonin, N1-methylnicotinamide, niacin, vitamin B3.

Introduction

Neuroendocrine tumors (NETs) are rare tumors, with an incidence of 2.4/100,000/year in the Netherlands. NETs derive from enterochromaffin cells and have the ability to produce various biogenic amines and polypeptide hormones of which serotonin is the most prominent [1]. Patients with serotonin-producing NET, in the past referred to as 'carcinoids', can suffer from a carcinoid syndrome characterized by flushing, diarrhea, asthma-like symptoms (e.g. wheezing) and valvular heart disease [1].

Serotonin is derived from the essential amino acid tryptophan, which plays an important role in the regulation of growth, mood, behavior and immune responses [2]. In healthy persons, only 1% of the tryptophan pool is used for serotonin production while the major part is metabolized by the kynurenine pathway to NAD⁺ (metabolically active form of niacin). In patients with a serotonin-producing NET, metabolism of tryptophan is diverted to the serotonin pathway. This can lead to the utilization of 60% of the tryptophan pool for serotonin synthesis in the tumor [3-5]. This derangement may then result in tryptophan and/or niacin (vitamin B3) depletion. A variety of symptoms and complaints related to changes in the skin, gastrointestinal tract and nervous system can be observed with tryptophan and/or niacin deficiency. Best known is pellagra, which is characterized by 'the four Ds': dermatitis, diarrhea, dementia and death [6-8]. The latter may occur in cases of untreated severe niacin and/or tryptophan deficiency. Nowadays, pellagra is rarely seen in the Western world. It is, however, still present in developing countries or individuals at risk as a consequence of malnourishment, such as chronic alcoholics, and patients with gastrointestinal disease or human immunodeficiency virus. Moreover, it can be induced by drugs (e.g. isoniazid, which affects tryptophan metabolism). Administration of niacin rapidly and effectively reduces symptoms and complaints in these persons [6-9].

In patients with serotonin-producing NETs, pellagra and other symptoms suggestive of tryptophan and/or niacin deficiency are reported [3,4,10-12]. Niacin supplementation resulted in a quick and substantial improvement in the symptoms of these patients [4,11]. However, there is scarcely any literature available regarding the systematical assessment of the niacin status in patients with serotonin-producing NET. A study performed in 1957 assessed the niacin metabolite urinary N1-methylnicotinamide (N1-MN) in 4 serotonin-producing NET patients and found that patients with the highest serotonin production had the lowest urinary N1-MN levels [13]. This finding suggests a low niacin status in patients with excessive serotonin-producing NET. More recently, the niacin status in newly diagnosed carcinoid patients, with (n = 36) and without (n = 32) carcinoid syndrome, was analyzed by measuring biologically active forms of niacin, NAD⁺ and NADP⁺ in whole blood [14]. Among the patients with carcinoid syndrome, 28% were biochemically niacin deficient, including 1 patient with signs of pellagra.

Measurement of the 24-hour output of urinary N1-MN is a reliable marker for niacin

deficiency and niacin status after supplementation. In contrast, assessment of the other niacin metabolite, N1-methyl-2-pyridone-5-carboxamide (2-pyr), in 24-hour urine or the ratio of 2-pyr/N1-MN are less accurate measures [15].

The aim of this study was to assess the niacin status by measurement of urinary N1-MN in serotonin-producing NET patients with tryptophan deficiency and/or associated symptoms, who therefore received niacin supplementation. The niacin status was serially assessed before and after supplementation to examine the effectiveness of niacin supplementation.

Materials and methods

Patients

Between January 2006 and February 2014, we identified serotonin-producing NET patients (tumor grades 1 and 2, according to the World Health Organization 2010 classification) under treatment at the Department of Medical Oncology of the University Medical Center Groningen, the Netherlands. Patients who had received niacin supplementation based on low plasma tryptophan levels ($<40 \mu\text{mol/l}$) and/or pellagra-like symptoms, as part of standard care, were included. Assessment of plasma tryptophan levels is part of the standard biochemical evaluation in serotonin-producing NETs in the above-mentioned NET referral center. The diagnosis of serotonin-producing NET was based on increased urinary 5-hydroxyindolacetic acid (5-HIAA; $>3.8 \text{ mmol/mol creatinine}$) [16]) secretion and/or platelet serotonin ($>5.4 \text{ nmol}/10^9 \text{ platelets}$ [16]) level. Histological analysis of the tumor tissue of the patients was centrally reviewed by a dedicated NET pathologist (G.K.-U.).

For the assessment of the 24-hour output of N1-MN in patients, residual archival urine was used. This does not interfere with patient care nor does it involve the physical involvement of the patient. Therefore, no ethical approval is required according to the Dutch legislation (the Medical Research Involving Human Subjects Act) [17].

Healthy individuals ($n = 133$) participating in the LifeLines cohort study [18] served as controls.

The LifeLines cohort study was approved by the Local Medical Ethics Committee of the University Medical Center Groningen, the Netherlands. All participating healthy individuals gave their written informed consent.

Laboratory methods and (pre-)analytical procedures

Presupplementation tryptophan levels in patients were assessed in platelet-rich plasma samples. In case of a missing plasma tryptophan level at the start of niacin supplementation, we used the nearest available plasma tryptophan level before the start

of niacin supplementation. Platelet-rich plasma was collected most often in a nonfasting state during routine outpatient visits. It was obtained by centrifugation at 120 g for 30 min. All platelet-rich plasma was pipetted into a plastic tube and carefully mixed; 0.5 ml served for thrombocyte counting and the remaining plasma was stabilized with a mixture of ethylenediaminetetraacetic acid (EDTA) and ascorbic acid and frozen at -20°C until tryptophan and serotonin analysis by high-performance liquid chromatography fluorometry [19].

Niacin status was evaluated by measuring the 24-hour output of urinary N1-MN.

For the assessment of the niacin status before supplementation, 24-hour urine samples collected before the start of niacin supplementation and the nearest available samples by the assessment of the presupplementation plasma tryptophan level were selected. Patients collected 24-hour urine for 5-HIAA assessment the day before their routine clinic visit. Urine was stabilized with 250 mg EDTA and ascorbic acid mixture (1:1). After mixing, part of the urine was used for routine 5-HIAA analysis [20] and residual material was frozen at -80 °C. N1-MN was analyzed by a newly developed liquid chromatography-tandem mass spectrometry method (LC-MS/MS). In short, 50 µl urine was pipetted into a 96-well plate. Internal standard 1-methylnicotinamide-d3 in acetonitrile was added. Subsequently, the plate was vortexed and calibrators and standards were diluted with acetonitrile. Finally, 5 µl was injected onto an LC-MS/MS system (Spark Holland, the Netherlands) using a Phenomenex Luna HILIC column (2.1 × 150 mm, 3 µm) and a tandem mass spectrometer Xevo TQ-MS (Waters, Milford, Mass., USA) in the selective reaction monitoring mode for mass spectrometric detection. Intra-assay (n = 20) and interassay (n = 12) imprecision was <4% at 3 different N1-MN levels in pooled urine. The quantification limit of N1-MN in urine was 0.170 µmol/l.

Reference ranges for N1-MN in 24-hour urine were established by analyzing 133 urine samples collected from healthy individuals. Reference intervals were calculated using Analyse-it for Excel (Analyse-it Software, Ltd., Leeds, UK).

Statistical analysis

All continuous variables were checked for normality of distribution using Q-Q plots and goodness-of-fit tests. In case of skewed distributions, medians and ranges are presented, and the Mann-Whitney U test was used to compare the significance of the differences in means. Spearman rank correlation analyses were carried out to determine correlations between continuous variables. All tests were performed two-sided, and p values <0.05 were considered significant. Analyses were done using the software package SPSS, version 22 for Windows (SPSS, Inc., Chicago, Ill., USA).

Results

Characteristics of patients and healthy controls

Patient characteristics are summarized in table 1. For this study, we identified 42 patients with a mean age of 64 years (range 41-80) at the start of niacin supplementation. As healthy controls, 52 males (39%) and 81 females (61%) with a median age of 45 years (range 20-79) were used. Most patients received prior treatment including palliative surgical resections and somatostatin analogue and/or interferon. The small intestine was the most frequent site of the primary tumor. All patients had metastatic disease. The mean dose of daily oral niacin supplementation was 144 mg (range 5-300).

Table 1. Patients characteristics (n = 42)

Gender	
Male	20 (48)
Female	22 (52)
Mean age at diagnosis NET, years	60 ± 11
Mean age at start niacin supplementation, years	64 ± 10
Location primary tumor	
Lung	2 (5)
Small intestine	21 (50)
Unknown	18 (43)
Other	1 (2)
Grade NET	
Grade 1	28 (67)
Grade 2	7 (16)
No histological tumor sample available	4 (10)
Insufficient histological tumor sample available for grading	3 (7)
Disease stage	
Stage 4	42 (100)
Antitumor treatment for NET before the start of niacin supplementation	
No	8 (19)
Yes	34 (81)
Type of antitumor treatment before the start of niacin supplementation	
Somatostatin analogue	27 (79)
Palliative surgery	23 (65)
Interferon	11 (32)
Everolimus	4 (12)
Chemotherapy	1 (3)
Peptide receptor radionuclide therapy	3 (9)
Liver embolization	1 (3)
Radiotherapy	4 (12)
Total of different antitumor treatments before the start of niacin supplementation	
1 treatment	12 (35)
2 treatments	12 (35)
3 treatments	3 (9)
≥4 treatments	7 (21)
Type of niacin supplementation	
Nicotinamide	36 (86)
Vitamin B complex	3 (7)
Vitamin B complex forte	2 (5)
Multivitamin	1 (2)

Data are expressed as number with percentages in parentheses or means ± SD.

Tryptophan and niacin status

Prior to the start of niacin supplementation, the mean plasma tryptophan level in patients was $31.8 \pm 9.7 \mu\text{mol/l}$ (range 8.3-51.0). The plasma tryptophan level was below the lower limit of normal (reference value 40-70 $\mu\text{mol/l}$ [19]) in 33 out of 42 (79%) patients. Among patients with normal plasma tryptophan levels, 7 had previous plasma tryptophan levels below the lower limit of normal, 1 had a low normal plasma tryptophan level with complaints of niacin deficiency, and 1 reported niacin deficiency-associated complaints with previous low plasma tryptophan levels. The majority (79%) of plasma tryptophan levels were assessed within 3 months before the start of niacin supplementation (range 0 days to 14 months).

Healthy controls ($n = 133$) were divided into age groups (decades) to examine if urinary N1-MN levels were influenced by age. Mean urinary N1-MN levels did not differ among these age groups ($p < 0.142$). Thereby, the healthy controls could serve as reference for urinary N1-MN levels. Calculated reference ranges for N1-MN in 24-hour urine in healthy controls were 17.3-115.0 $\mu\text{mol}/24 \text{ h}$.

In 74% of the patients ($n = 31/42$), 24-hour urine for the assessment of presupplementation urinary N1-MN levels was available within 1 week of presupplementation plasma tryptophan level (range 0 days to 4 months). Before supplementation, the median urinary N1-MN level of patients was lower (17.9 $\mu\text{mol}/24 \text{ h}$, range 2.6-70.3) compared to that of healthy controls (43.7 $\mu\text{mol}/24 \text{ h}$, range 9.5-169.3, $p < 0.0001$; fig. 1). Based on these reference values, 19 out of 42 (45%) patients had a deficient niacin status at baseline. Among these 19 deficient patients, 15 patients also had a plasma tryptophan level below normal at baseline. The 4 niacin-deficient patients without reduced plasma tryptophan levels had plasma tryptophan levels in the lower range of normal (range 40.5-51 $\mu\text{mol/l}$). Patients with plasma tryptophan levels below normal ($n = 33$) did not have lower N1-MN levels before supplementation (median 17.9 $\mu\text{mol}/24 \text{ h}$, range 2.6-70.3) compared to patients with sufficient tryptophan levels ($n = 9$; median 25.1 $\mu\text{mol}/24 \text{ h}$, range 10.1-64.9, $p = 0.483$).

In 34 patients, 24-hour urines were available for niacin status assessment after supplementation. After supplementation, the N1-MN level increased to above normal levels (median 103.3 $\mu\text{mol}/24 \text{ h}$, range 7.4-545.3) compared to calculated reference values (Fig. 2). In the patients with a deficient niacin status before supplementation ($n = 14$, 5 missing data), the N1-MN level increased to high normal (median 55.5 $\mu\text{mol}/24 \text{ h}$, range 7.4-489.0) after supplementation. Eventually, 86% of the niacin-deficient patients ($n = 12/14$) had a normal niacin status after supplementation.

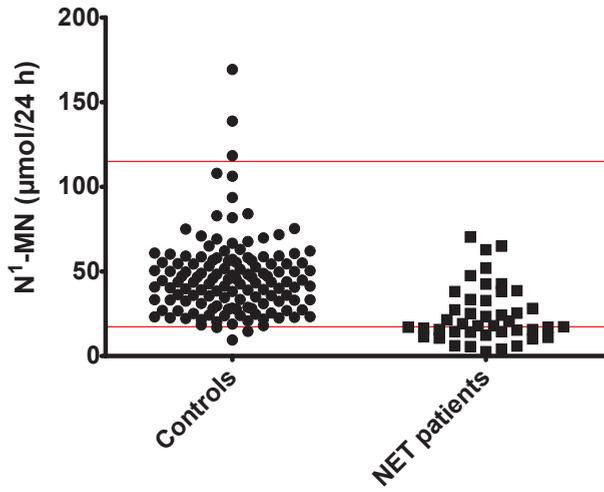


Figure 1. Urinary N¹-MN levels in healthy controls (n = 133) and before niacin supplementation in serotonin-producing NET patients (n = 42).

The red lines represent calculated reference ranges for urinary N¹-MN levels (17.3-115.0 µmol/24 h).

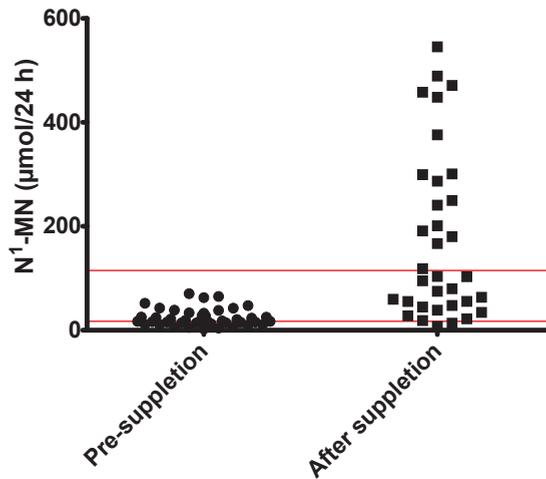


Figure 2. Urinary N¹-MN levels before (n = 42) and after niacin supplementation in serotonin-producing NET patients (n = 34).

The red lines represent calculated reference ranges for urinary N¹-MN levels (17.3-115.0 µmol/24 h).

Relationship serotonin production, tryptophan level and niacin status

At baseline, the median serotonin concentration in platelets was 20.0 nmol/10⁹ (range 5.1-44.0), and the median 5-HIAA excretion in urine was 15.0 mmol/mol creatinine (range 2.2-248.9). Urinary 24-hour excretion of 5-HIAA, the metabolite of serotonin, is a measure for serotonin production in NET patients. Niacin-deficient patients (n = 19) did not have a higher urinary 5-HIAA level (median 42.6 mmol/mol creatinine, range 2.2-234.2) compared to patients with a sufficient niacin status (n = 23, median 11.2 mmol/mol creatinine, range 2.2-248.9, p = 0.069). There was no difference in the 5-HIAA level in urine between patients with low plasma tryptophan levels (n = 33, median 17.1 mmol/mol creatinine, range 2.6-248.9) and patients with normal plasma tryptophan levels (n = 9, median 11.4 mmol/mol creatinine, range 2.2-147.4, p = 0.541).

To examine whether increased serotonin production leads to tryptophan and niacin deficiency, we assessed the correlation of urinary 5-HIAA levels with the tryptophan level and niacin status before the start of niacin supplementation (table 2). 5-HIAA levels in 24-hour urine showed a negative correlation with niacin status (p = 0.022, r = -0.352), indicating that before the start of niacin supplementation, NET patients with a higher serotonin production had a lower niacin status. The urinary 5-HIAA level did not correlate with the plasma tryptophan level (p = 0.183).

In the 19 patients with niacin deficiency at baseline, the urinary 5-HIAA level did not correlate with the plasma tryptophan level (p = 0.932) or the niacin status (p = 0.715). In these patients, the plasma tryptophan level correlated with the niacin status (p = 0.022, r = 0.522).

Table 2. Correlations between biochemical parameters (Spearman's rho)

	All patients (n = 42)			Niacin-deficient patients (n = 19)		
	Plasma tryptophan	Urine 5-HIAA	Urine N ¹ -MN	Plasma tryptophan	Urine 5-HIAA	Urine N ¹ -MN
Plasma tryptophan¹	1	-0.209	0.275	1	-0.021	0.522*
Urine 5-HIAA²	-0.209	1	-0.352*	-0.021	1	0.090
Urine N¹-MN³	0.275	-0.352*	1	0.522*	0.090	1

¹ in µmol/l, ² in mmol/mol creatinine, ³ in µmol/24 h

* p < 0.05

Discussion

This is the first study which biochemically evaluated the effectiveness of niacin supplementation in serotonin-producing NET patients with niacin deficiency. Niacin supplementation was effective in normalizing the niacin status in most of these patients. In this study, excessive serotonin production defined by elevated urinary 5-HIAA levels was related to a lower niacin status before the start of niacin supplementation. The difference in urinary 5-HIAA levels between niacin-deficient and niacin-sufficient patients did not reach the significance level, which may be due to the small number of patients. In contrast to earlier findings [19], the plasma tryptophan level was not related to excessive serotonin production. Also, in patients with a too low niacin status, no relation was found for serotonin production and plasma tryptophan level or niacin status. This may be explained by the fact that the plasma tryptophan level and the niacin status are influenced by several factors, such as dietary intake, malabsorption and drug interactions, which may have affected the findings in this study. NAD⁺ is not only de novo synthesized from tryptophan but can also be synthesized via salvage pathways from precursors in the diet, such as nicotinamide and nicotinic acid [21]. Another explanation may be found in the retrospective design of the study. Plasma tryptophan levels were not assessed nearby the 24-hour urine collection for 5-HIAA and N1-MN levels at the start of the niacin supplementation in all patients, which may have interfered in the above-mentioned examined relations.

The plasma tryptophan level is no accurate predictor of niacin status. Eighteen out of 42 patients had low plasma tryptophan levels with adequate niacin status, and 4 niacin-deficient patients had above normal plasma tryptophan levels. Assessment of plasma tryptophan concentrations in 26 healthy individuals showed a significant biological variation during the day with significant increases in plasma tryptophan concentrations after consumptions of meals [22]. Because of the biological variation and the need for drawing blood in a fasting state, it is not accurate and practical to use the plasma tryptophan level for the assessment of the niacin status. Therefore, assessment of the niacin status and the start of niacin supplementation should be based on the resultant of the deficiency, namely the urinary N1-MN level. Fourteen percent of the niacin-deficient patients in our study were still deficient after niacin supplementation. This might be due to the compliance with vitamin supplementation which may not have been accurate at all times. Daily oral administration of niacin is required since it is a water-soluble vitamin and thereby not stored in the body [9]. The prevalence of pellagra in serotonin-producing NET patients is about 5% [12] and especially reveals during the advanced stage of the disease [10]. Niacin deficiency might be underestimated in patients with a serotonin-producing NET. Symptoms of niacin deficiency can resemble and coincide

with symptoms due to serotonin production of NET, such as diarrhea, which impairs the recognition of clinical niacin deficiency. Also, treating physicians may not be aware of niacin deficiency and accompanying pellagra since diagnosing, preventing or treating these conditions have received little attention in earlier literature and current available guidelines for treating NET patients. Given the impact of NET on the patient's quality of life [23,24] and the relatively long-term survival, a valuable additional goal in the care for NET patients should be preventing clinical niacin deficiency such as pellagra.

Regular biochemical evaluation including 24-hour urinary excretion of 5-HIAA is standard practice to evaluate the effect of therapy and tumor progression in serotonin-producing NET patients [25,26]. Given our findings, we consider it of clinical interest to include urinary N1-MN in this biochemical assessment to facilitate early diagnosis and prevention of niacin deficiency. In case of biochemically proven niacin deficiency, supplementation of niacin can easily and effectively treat niacin deficiency and prevent accompanying symptoms.

A prospective study is warranted to assess the prevalence of niacin deficiency and to evaluate the most effective method of niacin supplementation for treating biochemical and clinical niacin deficiency among serotonin-producing NET patients.

Acknowledgments

None.

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