Neuroendocrinology

Research Article

Neuroendocrinology 2025;115:411–421
DOI: 10.1159/000543658

Received: September 18, 2024
Accepted: January 12, 2025
Published online: January 21, 2025

Impact of Indoleamine 2,3-Dioxygenase Enzyme Activity in Neuroendocrine Tumors

Camilla Mancini[a, b] Giulia Pecora[a] Gerardo Salerno[c] Luana Lionetto[d] Donatella De Bernardini[d] Giuseppe Costanzi[d] Saverio Gabrielli[a] Domenico Veroli[c] Vincenzo Visco[c] Maurizio Simmaco[e] Virginia Zamponi[a] Rossella Mazzilli[a] Antongiulio Faggiano[a]

[a]Unit of Endocrinology, Department of Clinical and Molecular Medicine, Sant'Andrea University Hospital, ENETS Center of Excellence, Sapienza University of Rome, Rome, Italy; [b]Department of Molecular Medicine, Sapienza University of Rome, Rome, Italy; [c]Laboratory of Clinical Biochemistry, Department of Clinical and Molecular Medicine, Sant'Andrea University Hospital, Sapienza University of Rome, Rome, Italy; [d]Mass Spectrometry Section, Clinical Biochemistry Laboratory, Sant'Andrea University Hospital, Rome, Italy; [e]Department of Neuroscience, Mental Health and Sensory Organs, Faculty of Medicine and Psychology, Sapienza University of Rome, Rome, Italy

Keywords
Neuroendocrine tumors · Indoleamine 2,3-dioxygenase · Tryptophan · Kynurenine · Cytokines · Immunotherapy

Abstract

Introduction: Indoleamine 2,3-dioxygenase (IDO) converts L-tryptophan (T) to L-kynurenine (K) resulting in an immunosuppressive microenvironment. The aim of the current study was to evaluate in patients with neuroendocrine tumor (NET) (1) T and K concentrations; (2) correlation with clinical outcome; (3) relationship between IDO activity and inflammatory cytokines. ***Methods:*** A cross-sectional study was performed to investigate the IDO pathway in patients in follow-up for NET. Clinicopathological features, serum levels of K and T through liquid chromatography, and serum assay of cytokines (IL-6, IL-10, IL-17A, IL-22, IL-23, TNF-α) through MAGPIX were evaluated. ***Results:*** Seventy-eight NET patients were enrolled (66 lung, 12 pancreatic): 69.2% were in postoperative remission, 14.1% in stable disease, and 16.7% in disease progression. T was significantly lower in patients older than 65 years ($p = 0.003$). K and T were significantly lower in patients with progression ($p = 0.03$, $p = 0.004$, respectively). T was an independent predictor factor of progression in multivariable analysis ($p = 0.041$). A cutoff of 7.74 μg/mL significantly differentiates patients with progression and those with stable disease. IL-6 and IL-10 were significantly associated with tumor progression in univariate analysis ($p = 0.005$, $p = 0.001$, respectively) but not in the multivariable analysis. A statistically significant negative correlation was found between T and IL-10 ($r = -0.366$, p value = 0.04). ***Conclusion:*** The K/T pathway may play a role as a potential predictor of tumor progression in NET. These findings need to be validated in large prospective studies investigating its metabolites as both prognostic and predictive factors for treatment response.

karger@karger.com
www.karger.com/nen

Correspondence to:
Antongiulio Faggiano, antongiulio.faggiano@uniroma1.it

Introduction

Indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO or TDO2) are rate-limiting enzymes, involved in more than 95% of tryptophan (T) catabolism along with the kynurenine (K) pathway. T is an essential amino acid in humans, serving multiple important functions, and is critical for the synthesis of key neuroendocrine mediators, such as serotonin, and the regulation of both local and systemic cell-mediated immunity [1]. Induction of IDO leads to the production of a group of metabolites known as "kynurenines" [1], which are recognized for their ability to suppress T-cell function and promote T-cell apoptosis [2, 3]. Moreover, T depletion stimulates stress mechanisms, through the activation of the general control non-derepressible 2 kinase, enhancing T-cell immunosuppressive signaling by causing T-cell G1 cycle arrest and Fas-mediated apoptosis [4, 5]. Some malignant solid tumors use IDO-induced immunosuppression as one of the immune evasion mechanisms [1, 5–9]; therefore, a higher IDO activity may be linked to a worse prognosis [9–13]. For this reason, IDO activity could be a tool to monitor disease activity and response to therapy in various malignant solid malignancies.

Neuroendocrine neoplasms (NEN) represent a heterogeneous group of malignancies originating from neuroendocrine cells distributed throughout the body [14]. They can arise in various organs but more commonly in the gastroenteropancreatic tract and lungs [15–18]. The main histotype is neuroendocrine tumors (NETs) exhibit considerable heterogeneity in their clinical presentation, histological features, and biological behavior, posing significant challenges for diagnosis and management [19–23]. In this regard, the pathological classification of NEN needs to be periodically updated and new tissue and circulating biomarkers tested and validated. Finally, imaging techniques still represent a challenge for clinicians, especially for follow-up, which needs to be standardized and adapted to the different scenarios [24]. Despite their relatively indolent course compared to other malignancies, a subset of NET may manifest aggressive behavior, leading to metastatic spread and worse clinical outcomes [25].

To date, the study of the tumor microenvironment (TME) has become crucial in various types of tumors [26], with the aim of identifying new therapeutic targets [27]. Immune cells such as B and T cells, NK cells, mast cells, dendritic cells, and macrophages infiltrate NET and create an immunosuppressive environment through various mechanisms [28–35]. The typical "immune contexture" of NET, characterized by poor lymphocyte infiltration and often referred to as a "cold" tumor [36, 37], lacks the features associated with response to immune checkpoint inhibitors (ICIs), which induce IDO and TDO expression [38]. To date, a number of IDO and TDO inhibitors have been tested into clinical trials with or without ICIs in patients with solid tumor [39]. Specifically, in advanced solid tumors, such as melanoma, bladder and kidney cancers, BMS-986205 [40] has been evaluated with nivolumab to target the K/T pathway to overcome immunosuppression. This approach aims to modulate immune responses, making the TME more responsive to immunotherapy. The combination of epacadostat [41] with pembrolizumab in early phase I/II studies appeared promising but was not effective in larger trials, highlighting the complexity of modulating the immune milieu [42]. New approaches like IDO1-targeted peptide vaccines are also being explored to enhance antitumor immune responses [43, 44]. These approaches could be particularly relevant for NET as they aim to activate immune cells in environments typically resistant to conventional ICIs. Other trials are ongoing for different tumor types, focusing on refining strategies of association therapies [45–47]. In this setting, a dynamic interplay between NET cells and the TME regulates the growth and tumor progression, and intensive research is needed to exploit the vulnerabilities of such molecular interactions. Several evidence suggested that the dysregulated activity of IDO and the associated establishment of an immunosuppressive TME may be influenced by various cytokines and signaling pathways [40–44]. In this regard, chronic inflammation appears to play a critical role in the development of these tumors through the network of different cytokines and growth factors such as TNF-α, IL-2, IL-6, IL-8, IL-1β [45, 46]. For instance, IL-6 can stimulate IDO expression in certain inflammatory conditions [47], IL-10 can increase IDO expression, particularly in immune-regulating settings, and can suppress the activation of the immune system [48], while IL-17A together with IL-22 are primarily involved in inflammatory immune responses, often associated with pathological conditions [47–49]. Finally, IL-23 and TNF-alpha can regulate IDO expression, particularly in the context of inflammatory and immune processes [48]. In light of these findings, understanding the interplay between IDO activity, TME, and tumor behavior could help to identify novel therapeutic strategies and prognostic markers in NET. While the role of IDO in immune evasion has been extensively studied in other cancers, its significance in NET remains poorly understood. Therefore, this study aimed to evaluate IDO enzymatic activity,

as expressed by the serum concentrations of K and T as well as their *ratio*, and its prognostic role in NET patients. Furthermore, the relationship between IDO activity and inflammatory cytokines was also investigated.

Methods

Sample Cohort and Clinical Data Evaluation

This is a cross-sectional study enrolling patients with a diagnosis of NET in follow-up at the ENETS center of excellence Sant'Andrea Hospital. Inclusion criteria were age≥18 years; histologically documented diagnosis of NET; Eastern Cooperative Oncology Group (ECOG) performance status ≤2; adequate cardiac, pulmonary, renal, liver, and bone marrow function; written informed consent. Exclusion criteria were histology of neuroendocrine carcinoma or mixed neuroendocrine/epithelial tumor; unknown primary site of NET; genetic syndrome; autoimmune disease; symptomatic interstitial lung disease; systemic immunosuppressive treatment; prior treatment with immune-stimulatory antitumor agents including checkpoint-targeted agents. Baseline clinicopathological data included primary tumor site, tumor stage (AJCC/UICC TNM classifications), tumor grade, ki67 index, disease status, NET-related endocrine syndromes.

Histological diagnosis was based on conventional hematoxylin-eosin staining and immunohistochemistry for neuroendocrine markers, according to the 2022 WHO classification of NEN [50, 51]. Disease status was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST, version 1.1).

Each study participant who accepted to participate in the study willingly provided written informed consent. The study adhered to the ethical standards outlined in the Declaration of Helsinki guidelines and the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines and was approved by Sapienza University Ethics Committee (RIF CE 6648/2022).

Kynurenine and Tryptophan Serum Assessment

Blood samples were collected, centrifuged to obtain serum samples, and stored at −20°C until analysis. Fifty microliters of serum sample was treated using 50 µL of deproteinizing solution, consisting of 4% aqueous solution of trichloroacetic acid. The mixture was mixed for 30 s and centrifuged at 14,000 rpm for 20 min. Ten microliters of clean supernatant was injected into the chromatographic system. Compounds were detected using a liquid chromatography-tandem mass spectrometry analytical method [52]. Chromatographic separation of analytes was performed using an Agilent Liquid Chromatography System series 1100 (Agilent Technologies, USA), on an F5 column (100 × 2.1 mm, Kinetex 2.6 µm Polar C18, 100 Å, Phenomenex, CA, USA) equipped with a security guard pre-column (Phenomenex, Torrance, CA, USA). The mobile phase consisted of a solution of 0.1% aqueous formic acid (A) and 100% methanol (B); elution was performed at a flow rate of 400 µL/min, using an elution gradient. The mass spectrometry method was performed on a 3200 triple quadrupole system (Applied Biosystems, Foster City, CA, USA) equipped with a TurboIonSpray source. The detector was set in the positive ion mode. The instrument was set in the multiple reaction monitoring mode. Data were acquired and processed by the Analyst 1.5.1 Software [52].

Cytokines Serum Assessment

Cytokines serum assessment was conducted in a subgroup of 39 patients. This pilot analysis was carried out in a limited number of patients due to sample availability. Blood samples were centrifuged at 1,500 *g* (NEYA 10R centrifuge, REMI ELEKTROTECHNIK LTD, Vasai 401-208, India) for 10 min and an aliquot of 350 µL of plasma was stored at −80°C until processing. Interleukin IL-6, IL-10, IL-17A, IL-22, IL-23, and TNF-α were determined using a human cytokine/chemokine magnetic bead panel A (Milliplex MAP kit, Millipore Corp., Billerica, MA, USA) and measured by a MAGPIX Luminex instrument and xPONENT software (version 4.2, Luminex Corp., Austin, TX, USA) according to manufacturer's instruction. Samples were analyzed undiluted. Luminex Multiplex Bead Immunoassays are solid-phase sandwich immunoassays. Beads of defined spectral properties conjugated to analyte-specific capture antibodies and samples (including standards of known analyte concentration, control specimens, and unknowns) are pipetted into the wells of a filter bottom microplate and incubated. During this first incubation, analytes bind to the capture antibodies on the beads. After washing the beads, analyte-specific biotinylated detector antibodies are added and incubated with the beads. During this second incubation, the analyte-specific biotinylated detector antibodies recognize their epitopes and bind to the appropriate immobilized analytes. After removal of excess biotinylated detector antibodies, streptavidin conjugated to the fluorescent protein, R-phycoerythrin (streptavidin-RPE), is added and incubated. During this final incubation, the streptavidin-RPE binds to the biotinylated detector antibodies

associated with the immune complexes on the beads, forming a four-member solid-phase sandwich. After washing to remove unbound streptavidin-RPE, the beads are analyzed in a subgroup of 39 patients with the Luminex instrument. By monitoring the spectral properties of the beads and the amount of associated RPE fluorescence, the concentration of one or more analytes can be determined.

Statistical Analysis

All normally distributed variables were represented as mean and standard deviation (mean ± standard deviation); variables that did not follow a normal distribution were represented as median with Q1 and Q3 quartiles. Qualitative variables were summarized as frequencies and percentages. Group comparisons were conducted using either the Student's *t* test or Mann-Whitney test, as appropriate, for quantitative variables and utilizing Pearson's chi-square or Fisher's test, as appropriate, for qualitative variables. A logistic regression analysis was performed using each clinicopathological characteristic as the dependent variable and K, T, K/T *ratio*, and cytokines tested in the study as covariates.

A receiver operating characteristic analysis was also performed. These results were expressed as area under the curve and 95% CI.

The Pearson correlation coefficient (r) was calculated to quantify the strength and direction of the linear relationship between IDO enzyme activity parameters and cytokines assayed. The results were reported as correlation coefficients (r) with associated *p* values. All statistical analyses were performed using IBM Statistical Package for Social Sciences (SPSS) version 22 (IBM Corp., Armonk, NY) and p value <0.05 was considered statistically significant.

Results

A total of 78 patients with NET were enrolled (shown in Table 1), of which 26 (33.3%) were males and 52 (66.6%) were females. The cohort included 66 patients with lung NET (84.6%) and 12 patients with pancreatic NET (15.4%). Overall, 54 were in postoperative remission (69.2%) and 24 had advanced disease (30.8%): 11 of them had stable disease (14.1%), while 13 of them had progressive disease (16.7%). Among the cohort of patients, 51 (65.4%) patients undergone surgery, 6 (7.7%) patients received somatostatin analogs (SSAs), and 21 (26.9%) received a combination therapy (SSA + targeted therapy and/or chemotherapy and/or peptide receptor radionuclide therapy) (shown in Table 1).

The mean K/T *ratio* was 0.02 ± 0.02 in the whole population, the mean K was 0.18 ± 0.06 μg/mL, and the mean T was 8.28 ± 2.26 μg/mL (shown in Table 1). K and T serum levels were significantly lower in patients with disease progression compared to patients with stable disease ($p = 0.03$ and $p = 0.004$, respectively) (shown in Table 2; Fig. 1). Moreover, T levels were significantly lower in patients older than 65 years of age ($p = 0.003$) (shown in Table 2). No statistically significant differences of K and T serum concentrations as well as K/T *ratio* were found according to sex, primary site, tumor stage, tumor grade, and Ki67% index.

A logistic regression analysis was conducted to identify the association between K and T serum concentrations, as well as K/T *ratio*, with the clinicopathological parameters (shown in Table 3). At the univariate analysis, K and T serum concentrations were associated with tumor progression ($p = 0.032$ and $p = 0.008$, respectively). At the multivariate analysis, only T serum concentration remained significantly associated with progression ($p = 0.041$) (shown in Table 3). Furthermore, at receiver operating characteristic analysis, T serum level was a significant predictor of tumor progression (area under the curve 0.723; 95% CI 0.593; 0.853) (shown in Table 3), with an optimal threshold value for T of 7.74 μg/mL, with a sensibility of 69.2% and specificity of 61.5%. In a subgroup of 39 patients with NET, IL-6, IL-10, IL-17a, IL-22, IL-23 serum levels were measured. The serum levels were 2.50 [0.15–114.41], 2.90 [2.50–31.92], 12.00 [0.67–12.00], 0.16 [0.14–0.92], 0.91 [0.83–2.67] median [IQR], respectively (shown in Table 4). No statistically significant differences were found according to age, gender, primary site, tumor stage, tumor grade, Ki67% index, and disease status. At the univariate analysis, IL-6 and IL-10 serum concentrations were associated with tumor progression ($p = 0.005$, $p = 0.001$, respectively). However, these results were not confirmed at multivariable analysis.

The Pearson correlation analysis revealed a statistically significant negative correlation between T serum levels and IL-10 ($r = -0.366$, $p = 0.046$). No other significant correlations were observed.

Discussion

The clinical benefit of immunotherapy in NET remains less well defined compared to other cancer types due to the typical features of NET, such as variable/

Table 1. Characteristics of the study population

	Overall (n = 78)	Subgroup (n = 39)
Age, median [IQR], years	65 [56.0–74.0]	64 [55.0–72.5]
Sex, *n* (%)		
Male	26 (33.3%)	12 (30.8%)
Female	52 (66.7%)	27 (69.2%)
Tumor site, *n* (%)		
Lung	66 (84.6%)	35 (89.7%)
Pancreas	12 (15.4%)	4 (10.3%)
Tumor grade, *n* (%)		
1	42 (53.8%)	20 (51.3%)
2	31 (39.8%)	16 (41.0%)
3	5 (6.4%)	3 (7.7%)
Tumor stage, *n* (%)		
I	47 (60.2%)	24 (61.5%)
II	11 (14.1%)	4 (10.3%)
III	8 (10.3%)	4 (10.3%)
IV	12 (15.4%)	7 (17.9%)
Ki67 index %, median [IQR]	2.00 [1.00, 4.00]	2.00 [1.00, 5.5]
Disease status, *n* (%)		
Remission	54 (69.2%)	25 (64.1%)
Stability	11 (14.1%)	8 (25.5%)
Progression	13 (16.7%)	6 (15.4%)
Functional syndrome, *n* (%)		
Insulinoma	2 (2.6%)	1 (2.6%)
Carcinoid syndrome	1 (1.3%)	1 (2.6%)
Cushing syndrome	1 (1.3%)	0 (2.6%)
Therapy, *n* (%)		
Surgery	51 (65.4%)	26 (66.7%)
SSA	6 (7.7%)	4 (10.3%)
SSA + other therapies (PRRT, targeted therapy, chemotherapy)	21 (26.9%)	9 (23.1%)
Serum L-kynurenine, mean ± SD, μg/mL	0.18 (0.06)	0.22 (0.06)
Serum L-tryptophan, mean ± SD, μg/mL	8.28 (2.26)	8.89 (2.43)
K/T, mean ± SD	0.02 (0.02)	0.03 (0.02)

This table summarizes the demographic and clinic-pathological characteristics of the whole study population (n = 78) and the subgroup undergone cytokines assessment (n = 39). SSA, somatostatin analog; PRRT, peptide receptor radionuclide therapy; K, L-kynurenine; T, L-tryptophan.

heterogeneous site of origin and highly variable biological characteristics, ranging from well- to moderately differentiated and from low to high grade [53]. In addition, NETs are characterized by a cold TME, which means they are less responsive to immunotherapies. This results in a loss of features associated with response to ICIs, such as the expression of IDO and TDO, which are typically involved in modulating the immune response. The reduced expression of these enzymes in NET contributes to the lower efficacy of ICIs in these tumors compared to other cancers [38, 54].

Several lines of evidence suggest that a dysregulated IDO activity contributes to tumor immune evasion, immune tolerance, and the immunosuppressive TME. These processes can be influenced by a range of cytokines and signaling pathways [55–59]. To date, several IDO and TDO inhibitors have been investigated in clinical trials, either alone or in combination with PD-1 inhibitors, in patients with solid tumors [39]. In particular, patients with NET may derive benefit from combination of immunotherapies to overcome the challenges posed by this cold immune microenvironment. In addition, the specific

Table 2. K/T *ratio* and serum concentrations of L-kynurenine and L-tryptophan according to the clinicopathological features of NET

	L-kynurenine, mean ± SD	*p* value	L-tryptophan, mean ± SD	*p* value	K/T *ratio*, mean ± SD	*p* value
Age, median (IQR)						
≤65	0.18 (0.06)	0.50	9.05 (2.12)	**0.003**	0.02 (0.01)	0.15
>65	0.17 (0.07)		7.54 (2.16)		0.03 (0.02)	
Sex						
Male	0.17 (0.07)	0.82	8.13 (2.07)	0.45	0.02 (0.01)	0.83
Female	0.18 (0.06)		8.58 (2.62)		0.01 (0.02)	
Tumor site						
Lung	0.17 (0.04)	0.57	8.72 (2.42)	0.47	0.02 (0.01)	0.79
Pancreas	0.18 (0.07)		8.20 (1.79)		0.03 (0.02)	
Tumor stage						
I, II	0.18 (0.07)	0.40	8.44 (2.02)	0.96	0.02 (0.02)	0.58
III, IV	0.18 (0.07)		7.83 (2.99)		0.02 (0.01)	
Tumor grade						
1	0.19 (0.07)	0.87	7.89 (1.84)	0.21	0.03 (0.02)	0.17
2, 3	0.16 (0.06)		8.56 (2.58)		0.02 (0.01)	
Ki67 index %						
≤2	0.19 (0.07)	0.25	8.15 (2.06)	0.55	0.03 (0.02)	0.19
>2	0.17 (0.05)		8.49 (2.59)		0.02 (0.01)	
Disease status, *n*						
Stability	0.19 (0.07)	**0.03**	8.55 (2.23)	**0.004**	0.03 (0.02)	0.24
Progression	0.14 (0.06)		6.76 (1.77)		0.02 (0.01)	

K, L-kynurenine; T, L-tryptophan.

correlations between immunotherapies and IDO activity may differ depending on the context and type of pathology.

Interestingly, our results showed that most of the clinicopathological features investigated do not play a significant role in the variation of K, T, K/T *ratio*, and all cytokines, in agreement with the literature available to date [60]. On the other hand, age and tumor progression were significantly associated to T serum concentrations, being lower in older than younger subjects and in progressive than stable tumor. Tumor progression was also associated with lower serum values of K. The observed reduction in T levels in older patients is consistent with established findings on the effects of aging on T metabolism. Indeed, T is essential for many physiological processes, including protein synthesis and immune regulation; T depletion in older adults has been linked to immune dysfunction and heightened activity of the kynurenine pathway [61]. The findings of this study may reflect these broader patterns. A reduction in T levels may also contribute to disease progression, as suggested by the association between lower T levels and tumor progression in NET patients. These findings are consistent with existing literature and further support the role of altered T metabolism in both aging and disease progression [61]. In contrast, the K/T *ratio*, which reflects the activity of IDO enzyme, was not increased in patients with progressive disease compared to patients with stable disease. One potential explanation is that alternative metabolic pathways are enhanced in patients with progressive NET, resulting in lower T levels and also explaining the lower K levels found in these patients [62]. Among the alternative metabolic pathways targeting T, the serotonin synthesis may be upregulated in NET patients with tumor progression. However, this was not the objective of the present study. Anyway, given the frequent dysregulation of the serotonin pathway in NET, even in the absence of a classic carcinoid syndrome, it is reasonable to hypothesize that increased serotonin synthesis could represent a tumor escape mechanism. Indeed, serotonin is known to promote cell proliferation, angiogenesis, and metastasis, thereby facilitating tumor growth [63, 64]. Therefore, we might speculate that increased serotonin synthesis may provide an alternative mechanism to IDO activation in supporting tumor growth in patients with progressive disease. Dedicated studies are needed to elucidate the

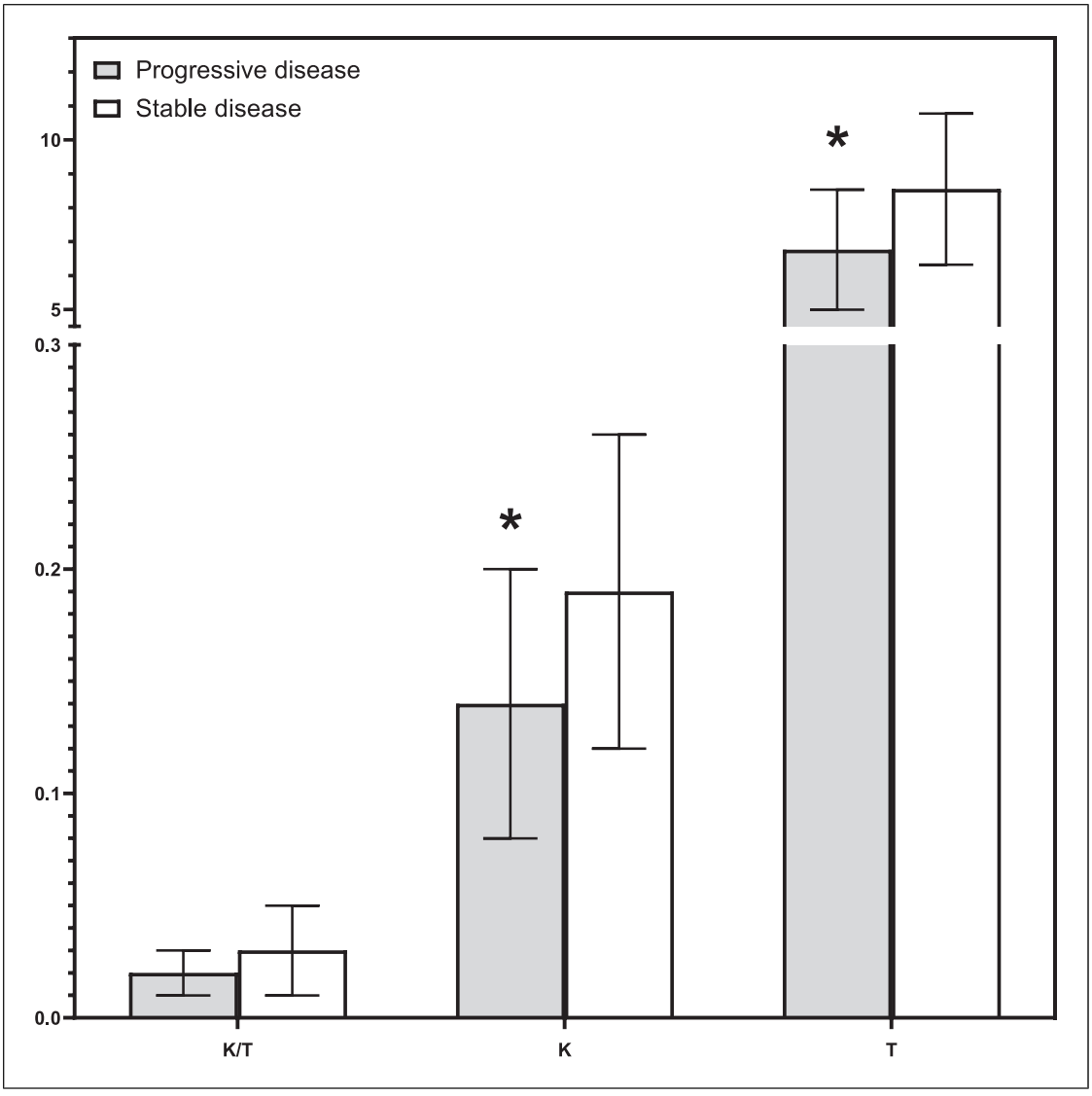

Fig. 1. L-kynurenine, L-tryptophan, and K/T ratio in NET patients with stable and progressive disease. The mean values of L-kynurenine (K), L-tryptophan (T), and their *ratio* (K/T) in patients with neuroendocrine tumors (NETs) accompanied by SDs. The data correspond to the last three rows of Table 3. A key finding illustrated by the figure is that T and K levels were significantly lower in patients with disease progression compared to those with stable disease (6.76 ± 1.77 vs. 8.55 ± 2.23, p value 0.004; 0.14 ± 0.06 vs. 0.19 ± 0.07, p value 0.03, respectively). This suggests a potential role of tryptophan depletion in disease progression, which could be explored as a prognostic indicator in NET.

relative roles of these different pathways and to identify novel targeted therapies.

A previous report found a significant correlation between elevated IDO activity, as suggested by increased K/T *ratio* and decreased patient survival [65]; interestingly, the authors described IDO activity as a potential prognostic marker in NET, highlighting that increased IDO activity, leading to T depletion and K accumulation, correlates with poorer clinical outcomes and higher mortality rates [65]. In contrast to this previous study, our data showed a significant reduction in the mean T value in patients with disease progression compared to those with stable disease, emphasizing the specific role of T levels. On the other hand, survival analysis in the present study was not reliable due to the extremely low death rate. An intriguing hypothesis is that the worst outcome is associated with K accumulation rather than to T depletion, thus explaining the different findings between the

Table 3. Predictive factors of disease progression in NET: univariate, multivariate, and ROC analysis

	Univariate	Multivariable		ROC analysis		
	p value	*p* value	95% CI	AUC	95% CI	*p* value
L-kynurenine (μg/mL)	**0.032**	–	–	–		–
L-tryptophan (μg/mL)	**0.008**	**0.041**	**0.474–0.984**	0.723	**0.593–0.853**	**0.011**
K/T *ratio*	0.304	–	–	–		–
IL-23	0.213	–	–	–	–	–
IL-6	**0.005**	–	–	–	–	–
IL-10	**0.001**	–	–	–	–	–
IL-17a	0.773	–	–	–	–	–
IL-22	0.281	–	–	–	–	–
TNF-α	0.839	–	–	–	–	–

K, L-kynurenine; T, L-tryptophan; AUC, area under the curve; ROC, receiver operating characteristic. Statistically significant data ($p < 0.05$) are shown in bold.

Table 4. Cytokines serum levels in a subgroup of patients with NET ($n = 39$)

	Patients ($n = 39$)
Serum IL-6, median [IQR]	2.50 [0.15–114.41]
Serum IL-10, median [IQR]	2.90 [2.50–31.92]
Serum IL-17a, median [IQR]	12.00 [0.67–12.00]
Serum IL-22, median [IQR]	0.16 [0.14–0.92]
Serum IL-23, median [IQR]	0.91 [0.83–2.67]
Serum TNF-alpha, median [IQR]	8.12 [6.67–14.51]

study from Pschowski study and the present one [65]. However, the smaller sample size of the current study should be also considered.

The evaluation of systemic therapies, such as SSAs, provides an opportunity to explore their potential effects on modulation of the K/T ratio. SSAs have anti-proliferative and immunomodulatory effects in NET. These effects occur through their action on somatostatin receptors, particularly SST2, affecting tumor cell signaling, angiogenesis, and immune responses [66]. However, to date no studies explored this specific effect of SSAs on IDO.

When cytokines were evaluated according to progression, IL-10 and IL-6 were significantly associated, although multivariable analysis did not confirm this relationship. These findings highlight the critical roles of specific factors such as interleukin in understanding and predicting the progression of NET, which can inform more effective treatment strategies [48]. Specifically, according to Munir et al. [48], we can speculate that IL-10 may suppress the activation of the immune system and therefore maintain immune system tolerance to the tumor. The negative correlation between T and IL-10 observed in our study could indicate that lower T levels might increase IL-10-mediated immune suppression [67].

Finally, when comparing IDO activity in NET with other malignancies reported in the literature, the K/T ratio was significantly lower in NET than in non-small cell lung carcinoma and squamous cell carcinoma of the head and neck (0.057 ± 0.035 and 0.055 ± 0.026, respectively) [68]. This suggests potential differences in IDO activity and its implications across different tumor types. Specifically, a previous study conducted on 26 patients with non-small cell lung carcinoma showed that higher kyn/trp ratio could predict resistance to anti-PD-1 treatment [68]. The comparative analysis underscores the tumor-specific IDO activity, highlighting that this pathway should be validated for each kind of tumor, to personalize the use of this tool in clinical management. The main limitation of the study is the small number of patients enrolled, mainly for cytokine analysis, which represent the preliminary results of a pilot study.

Furthermore, the heterogeneity of the tumor characteristics represents another limitation. However, this point is an intrinsic characteristic of NET, which encompasses different primary origins and histotypes.

Conclusion

Understanding the molecular pathways involved in IDO activity may shed light on the sensitivity to immunotherapy in these patients. T depletion seems to play a significant role in disease progression among patients with NET. These findings emphasize the importance of monitoring T metabolism and cytokine levels (IL-6 and IL-10) in the clinical management in patients with NET. Conversely, no significant associations were found between K, K/T *ratio*, or cytokine levels with gender, tumor site, stage, or grade. A larger cohort in a multicenter study, also including other biomarkers such as serotonin, should be encouraged to validate these observations.

Statement of Ethics

This study was carried out according to the Declaration of Helsinki guidelines and was approved by the local Ethics Committee (Sapienza University Ethics Committee [RIF CE 6648/2022]). All the patients enrolled in the study signed the informed consent form.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

Funding Sources

This study was funded by institutional research funds of the Department of Clinical and Molecular Medicine, Sapienza University of Rome (protocol number: AR2221816C49D308).

Author Contributions

Conceptualization: C.M., R.M., G.P., V.Z., and A.F.; data curation: C.M., R.M., G.S., V.V., D.V., D.D.B., S.G., G.C., and A.F.; formal analysis: R.M., C.M., G.P., L.L., S.G., and G.S.; funding acquisition: R.M., V.Z., M.S., and A.F.; investigation: C.M., G.P., R.M., V.Z., and A.F.; methodology: L.L., G.S., V.V, G.C., D.V., and D.D.B; project administration and resources: R.M., M.S., and A.F.; software: R.M., C.M., and G.S.; supervision: R.M., V.Z., M.S., and A.F.; writing – original draft: C.M., G.P., G.S., S.G., and L.L.; and writing – review and editing: R.M., V.Z., M.S., V.V., and A.F. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

The datasets generated and/or analyzed during the current study are not publicly available because they are the property of the institution. The data are available from the corresponding authors on reasonable request with prior authorization from the institution.

References

1 Moffett JR, Namboodiri MA. Tryptophan and the immune response. Immunol Cell Biol. 2003;81(4):247–65. https://doi.org/10.1046/j.1440-1711.2003.t01-1-01177.x
2 Opitz CA, Wick W, Steinman L, Platten M. Tryptophan degradation in autoimmune diseases. Cell Mol Life Sci. 2007;64(19–20):2542–63. https://doi.org/10.1007/s00018-007-7140-9
3 Puccetti P, Grohmann U. Ido and regulatory T cells: a role for reverse signalling and non-canonical NF-kappaB activation. Nat Rev Immunol. 2007;7(10):817–23. https://doi.org/10.1038/nri2163
4 Munn DH, Sharma MD, Baban B, Harding HP, Zhang Y, Ron D, et al. GCN2 kinase in T cells mediates proliferative arrest and anergy induction in response to indoleamine 2,3-dioxygenase. Immunity. 2005;22(5):633–42. https://doi.org/10.1016/j.immuni.2005.03.013
5 Fallarino F, Grohmann U, You S, McGrath BC, Cavener DR, Vacca C, et al. Tryptophan catabolism generates autoimmune-preventive regulatory T cells. Transpl Immunol. 2006;17(1):58–60. https://doi.org/10.1016/j.trim.2006.09.017
6 Sucher R, Kurz K, Weiss G, Margreiter R, Fuchs D, Brandacher G. Ido-mediated tryptophan degradation in the pathogenesis of malignant tumor disease. Int J Tryptophan Res. 2010;3:113–20. https://doi.org/10.4137/ijtr.s4157
7 Uyttenhove C, Pilotte L, Théate I, Stroobant V, Colau D, Parmentier N, et al. Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. Nat Med. 2003;9(10):1269–74. https://doi.org/10.1038/nm934
8 Austin CJD, Rendina LM. Targeting key dioxygenases in tryptophan-kynurenine metabolism for immunomodulation and cancer chemotherapy. Drug Discov Today. 2015;20(5):609–17. https://doi.org/10.1016/j.drudis.2014.11.007
9 Selvan SR, Dowling JP, Kelly WK, Lin J. Indoleamine 2,3-dioxygenase (Ido): biology and target in cancer immunotherapies. Curr Cancer Drug Targets. 2016;16(9):755–64. https://doi.org/10.2174/1568009615666151030102250
10 Schefold JC, Zeden J-P, Pschowski R, Hammoud B, Fotopoulou C, Hasper D, et al. Treatment with granulocyte-macrophage colony-stimulating factor is associated with reduced indoleamine 2,3-dioxygenase activity and kynurenine pathway catabolites in patients with severe sepsis and septic shock. Scand J Infect Dis. 2010;42(3):164–71. https://doi.org/10.3109/00365540903405768
11 Zeden JP, Fusch G, Holtfreter B, Schefold JC, Reinke P, Domanska G, et al. Excessive tryptophan catabolism along the kynurenine pathway precedes ongoing sepsis in critically ill patients. Anaesth Intensive Care. 2010;38(2):307–16. https://doi.org/10.1177/0310057X1003800213
12 Sadeghi M, Lahdou I, Daniel V, Schnitzler P, Fusch G, Schefold JC, et al. Strong association of phenylalanine and tryptophan metabolites with activated cytomegalovirus infection in kidney transplant recipients. Hum Immunol. 2012;73(2):186–92. https://doi.org/10.1016/j.humimm.2011.11.002
13 Fotopoulou C, Sehouli J, Pschowski R, Von Haehling S, Domanska G, Braicu E-I, et al. Systemic changes of tryptophan catabolites via the indoleamine-2,3-dioxygenase pathway in primary cervical cancer. Anticancer Res. 2011;31(8):2629–35.
14 Klöppel G. Neuroendocrine neoplasms: dichotomy, origin and classifications. Visc Med. 2017;33(5):324–30. https://doi.org/10.1159/000481390

15 Laffi A, Lania AGA, Ragni A, Di Vito V, Liccardi A, Rubino M, et al. Gastric neuroendocrine tumors (g-NETs): a systematic review of the management and outcomes of type 3 g-NETs. Cancers (Basel). 2023;15(8):2202. https://doi.org/10.3390/cancers15082202
16 Hofland J, Falconi M, Christ E, Castaño JP, Faggiano A, Lamarca A, et al. European Neuroendocrine Tumor Society 2023 guidance paper for functioning pancreatic neuroendocrine tumour syndromes. J Neuroendocrinol. 2023;35(8):e13318. https://doi.org/10.1111/jne.13318
17 La Salvia A, Persano I, Siciliani A, Verrico M, Bassi M, Modica R, et al. Prognostic significance of laterality in lung neuroendocrine tumors. Endocrine. 2022;76(3):733–46. https://doi.org/10.1007/s12020-022-03015-w
18 La Salvia A, Marcozzi B, Manai C, Mazzilli R, Landi L, Pallocca M, et al. Rachel score: a nomogram model for predicting the prognosis of lung neuroendocrine tumors. J Endocrinol Invest. 2024;47(10):2575–86. https://doi.org/10.1007/s40618-024-02346-x
19 Nicholson AG, Tsao MS, Beasley MB, Borczuk AC, Brambilla E, Cooper WA, et al. The 2021 WHO classification of lung tumors: impact of advances since 2015. J Thorac Oncol. 2022;17(3):362–87. https://doi.org/10.1016/j.jtho.2021.11.003
20 Nagtegaal ID, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, et al. The 2019 WHO classification of tumours of the digestive system. Histopathology. 2020;76(2):182–8. https://doi.org/10.1111/his.13975
21 Öberg K, Knigge U, Kwekkeboom D, Perren A; ESMO Guidelines Working Group. Neuroendocrine gastro-entero-pancreatic tumors: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2012;23(Suppl 7):vii124–30. https://doi.org/10.1093/annonc/mds295
22 Arrivi G, Verrico M, Roberto M, Barchiesi G, Faggiano A, Marchetti P, et al. Capecitabine and temozolomide (captem) in advanced neuroendocrine neoplasms (NENs): a systematic review and pooled analysis. Cancer Manag Res. 2022;14:3507–23. https://doi.org/10.2147/CMAR.S372776
23 Faggiano A. Long-acting somatostatin analogs and well differentiated neuroendocrine tumors: a 20-year-old story. J Endocrinol Invest. 2024;47(1):35–46. https://doi.org/10.1007/s40618-023-02170-9
24 Modica R, Benevento E, Liccardi A, Cannavale G, Minotta R, Di Iasi G, et al. Recent advances and future challenges in the diagnosis of neuroendocrine neoplasms. Minerva Endocrinol. 2024;49(2):158–74. https://doi.org/10.23736/S2724-6507.23.04140-4
25 Dasari A, Shen C, Halperin D, Zhao B, Zhou S, Xu Y, et al. Trends in the incidence, prevalence, and survival outcomes in patients with neuroendocrine tumors in the United States. JAMA Oncol. 2017;3(10):1335–42. https://doi.org/10.1001/jamaoncol.2017.0589
26 Wang J-J, Lei K-F, Han F. Tumor microenvironment: recent advances in various cancer treatments. Eur Rev Med Pharmacol Sci. 2018;22(12):3855–64. https://doi.org/10.26355/eurrev_201806_15270
27 Cives M, Silvestris F, Pelle E, Quaresmini D, Rizzo FM, Tucci M. The tumor microenvironment in neuroendocrine tumors: biology and therapeutic implications. Neuroendocrinology. 2019;109(2):83–99. https://doi.org/10.1159/000497355
28 da Silva A, Bowden M, Zhang S, Masugi Y, Thorner AR, Herbert ZT, et al. Characterization of the neuroendocrine tumor immune microenvironment. Pancreas. 2018;47(9):1123–9. https://doi.org/10.1097/MPA.0000000000001150
29 Katz SC, Donkor C, Glasgow K, Pillarisetty VG, Gönen M, Espat NJ, et al. T cell infiltrate and outcome following resection of intermediate-grade primary neuroendocrine tumours and liver metastases. HPB Oxf. 2010;12(10):674–83. https://doi.org/10.1111/j.1477-2574.2010.00231.x
30 Cives M, Strosberg J, Al Diffalha S, Coppola D. Analysis of the immune landscape of small bowel neuroendocrine tumors. Endocr Relat Cancer. 2019;26(1):119–30. https://doi.org/10.1530/ERC-18-0189
31 Vikman S, Sommaggio R, De La Torre M, Oberg K, Essand M, Giandomenico V, et al. Midgut carcinoid patients display increased numbers of regulatory T cells in peripheral blood with infiltration into tumor tissue. Acta Oncol. 2009;48(3):391–400. https://doi.org/10.1080/02841860802438495
32 Aparicio-Pagés MN, Verspaget HW, Peña AS, Jansen JB, Lamers CB. Natural killer cell activity in patients with neuroendocrine tumours of the gastrointestinal tract; relation with circulating gastrointestinal hormones. Neuropeptides. 1991;20(1):1–7. https://doi.org/10.1016/0143-4179(91)90033-f
33 Soucek L, Lawlor ER, Soto D, Shchors K, Swigart LB, Evan GI. Mast cells are required for angiogenesis and macroscopic expansion of Myc-induced pancreatic islet tumors. Nat Med. 2007;13(10):1211–8. https://doi.org/10.1038/nm1649
34 Katsenelson NS, Shurin GV, Bykovskaia SN, Shogan J, Shurin MR. Human small cell lung carcinoma and carcinoid tumor regulate dendritic cell maturation and function. Mod Pathol. 2001;14(1):40–5. https://doi.org/10.1038/modpathol.3880254
35 Krug S, Abbassi R, Griesmann H, Sipos B, Wiese D, Rexin P, et al. Therapeutic targeting of tumor-associated macrophages in pancreatic neuroendocrine tumors. Int J Cancer. 2018;143(7):1806–16. https://doi.org/10.1002/ijc.31562
36 Wang L, Geng H, Liu Y, Liu L, Chen Y, Wu F, et al. Hot and cold tumors: immunological features and the therapeutic strategies. MedComm. 2023;4(5):e343. https://doi.org/10.1002/mco2.343
37 Khosravi G, Mostafavi S, Bastan S, Ebrahimi N, Gharibvand RS, Eskandari N. Immunologic tumor microenvironment modulators for turning cold tumors hot. Cancer Commun. 2024;44(5):521–53. https://doi.org/10.1002/cac2.12539
38 de Hosson LD, Takkenkamp TJ, Kats-Ugurlu G, Bouma G, Bulthuis M, de Vries EGE, et al. Neuroendocrine tumours and their microenvironment. Cancer Immunol Immunother. 2020;69(8):1449–59. https://doi.org/10.1007/s00262-020-02556-1
39 Atridia Pty Ltd. NCT03208959. A phase I, open-label, multicenter, non-randomized, dose-escalation study to evaluate the safety and tolerability of HTI-1090 in patients with advanced solid tumors. https://clinicaltrials.gov/study/NCT03208959#study-overview. 2017Aug
40 Bouma G, van Faassen M, Kats-Ugurlu G, de Vries EGE, Kema IP, Walenkamp AME. Niacin (vitamin B3) supplementation in patients with serotonin-producing neuroendocrine tumor. Neuroendocrinology. 2016;103(5):489–94. https://doi.org/10.1159/000440621
41 Weinlich G, Murr C, Richardsen L, Winkler C, Fuchs D. Decreased serum tryptophan concentration predicts poor prognosis in malignant melanoma patients. Dermatology. 2007;214(1):8–14. https://doi.org/10.1159/000096906
42 Schröcksnadel K, Wirleitner B, Winkler C, Fuchs D. Monitoring tryptophan metabolism in chronic immune activation. Clin Chim Acta. 2006;364(1–2):82–90. https://doi.org/10.1016/j.cca.2005.06.013
43 Kema IP, Schellings AM, Hoppenbrouwers CJ, Rutgers HM, de Vries EG, Muskiet FA. High performance liquid chromatographic profiling of tryptophan and related indoles in body fluids and tissues of carcinoid patients. Clin Chim Acta. 1993;221(1–2):143–58. https://doi.org/10.1016/0009-8981(93)90029-4
44 Azimnasab-Sorkhabi P, Soltani-Asl M, Yoshinaga TT, Zaidan Dagli ML, Massoco CDO, Kfoury Junior JR. Indoleamine-2,3 dioxygenase: a fate-changer of the tumor microenvironment. Mol Biol Rep. 2023;50(7):6133–45. https://doi.org/10.1007/s11033-023-08469-3
45 Vitale G, Carra S, Ferraù F, Guadagno E, Faggiano A, Colao A, et al. Gastro-enteropancreatic neuroendocrine neoplasms and inflammation: a complex cross-talk with relevant clinical implications. Crit Rev Oncol Hematol. 2020;146:102840. https://doi.org/10.1016/j.critrevonc.2019.102840
46 Ma Z-Y, Gong Y-F, Zhuang H-K, Zhou Z-X, Huang S-Z, Zou Y-P, et al. Pancreatic neuroendocrine tumors: a review of serum biomarkers, staging, and management. World J Gastroenterol. 2020;26(19):2305–22. https://doi.org/10.3748/wjg.v26.i19.2305
47 Sørensen RB, Hadrup SR, Svane IM, Hjortsø MC, Thor Straten P, Andersen MH. Indoleamine 2,3-dioxygenase specific, cytotoxic T cells as immune regulators. Blood. 2011;117(7):2200–10. https://doi.org/10.1182/blood-2010-06-288498

48 Munir S, Larsen SK, Iversen TZ, Donia M, Klausen TW, Svane IM, et al. Natural CD4+ T-cell responses against indoleamine 2,3-dioxygenase. PLoS One. 2012;7(4):e34568. https://doi.org/10.1371/journal.pone.0034568
49 Yu L, Li L. Potential biomarkers of atopic dermatitis. Front Med. 2022;9:1028694. https://doi.org/10.3389/fmed.2022.1028694
50 Borbath I, Pape U, Deprez PH, Bartsch DK, Caplin M, Falconi M, et al. ENETS standardized (synoptic) reporting for endoscopy in neuroendocrine tumors. J Neuroendocrinol. 2022;34(3):e13105. https://doi.org/10.1111/jne.13105
51 Rindi G, Mete O, Uccella S, Basturk O, La Rosa S, Brosens LAA, et al. Overview of the 2022 WHO classification of neuroendocrine neoplasms. Endocr Pathol. 2022;33(1):115–54. https://doi.org/10.1007/s12022-022-09708-2
52 Lionetto L, Ulivieri M, Capi M, De Bernardini D, Fazio F, Petrucca A, et al. Increased kynurenine-to-tryptophan ratio in the serum of patients infected with SARS-CoV2: an observational cohort study. Biochim Biophys Acta, Mol Basis Dis. 2021;1867(3):166042. https://doi.org/10.1016/j.bbadis.2020.166042
53 Albertelli M, Dotto A, Nista F, Veresani A, Patti L, Gay S, et al. Present and future of immunotherapy in neuroendocrine tumors. Rev Endocr Metab Disord. 2021;22(3):615–36. https://doi.org/10.1007/s11154-021-09647-z
54 Bowden M, Sicinska E, Kulke M, Loda M. Abstract 168: understanding the role of the carcinoid associated fibroblasts in the neuroendocrine tumor microenvironment. Cancer Res. 2014;74(19_Supplement):168. https://doi.org/10.1158/1538-7445.am2014-168
55 Bouma G, van Faassen M, Kats-Ugurlu G, de Vries EGE, Kema IP, Walenkamp AME. Niacin (vitamin B3) supplementation in patients with serotonin-producing neuroendocrine tumor. Neuroendocrinology. 2016;103(5):489–94. https://doi.org/10.1159/000440621
56 Weinlich G, Murr C, Richardsen L, Winkler C, Fuchs D. Decreased serum tryptophan concentration predicts poor prognosis in malignant melanoma patients. Dermatology. 2007;214(1):8–14. https://doi.org/10.1159/000096906
57 Schröcksnadel K, Wirleitner B, Winkler C, Fuchs D. Monitoring tryptophan metabolism in chronic immune activation. Clin Chim Acta. 2006;364(1–2):82–90. https://doi.org/10.1016/j.cca.2005.06.013
58 Kema IP, Schellings AM, Hoppenbrouwers CJ, Rutgers HM, de Vries EG, Muskiet FA. High performance liquid chromatographic profiling of tryptophan and related indoles in body fluids and tissues of carcinoid patients. Clin Chim Acta. 1993;221(1–2):143–58. https://doi.org/10.1016/0009-8981(93)90029-4
59 Azimnasab-Sorkhabi P, Soltani-Asl M, Yoshinaga TT, Zaidan Dagli ML, Massoco CDO, Kfoury Junior JR. Indoleamine-2,3 dioxygenase: a fate-changer of the tumor microenvironment. Mol Biol Rep. 2023;50(7):6133–45. https://doi.org/10.1007/s11033-023-08469-3
60 Yang P, Zhang J. Indoleamine 2,3-dioxygenase (Ido) activity: a perspective biomarker for laboratory determination in tumor immunotherapy. Biomedicines. 2023;11(7):1988. https://doi.org/10.3390/biomedicines11071988
61 Platten M, Nollen EAA, Röhrig UF, Fallarino F, Opitz CA. Tryptophan metabolism as a common therapeutic target in cancer, neurodegeneration and beyond. Nat Rev Drug Discov. 2019;18(5):379–401. https://doi.org/10.1038/s41573-019-0016-5
62 Badawy AA-B. The role of nonesterified fatty acids in cancer biology: focus on tryptophan and related metabolism. Biochim Biophys Acta Mol Cell Biol Lipids. 2024;1869(7):159531. https://doi.org/10.1016/j.bbalip.2024.159531
63 Balakrishna P, George S, Hatoum H, Mukherjee S. Serotonin pathway in cancer. Int J Mol Sci. 2021;22(3):1268. https://doi.org/10.3390/ijms22031268
64 Oronsky B, Ma PC, Morgensztern D, Carter CA. Nothing but NET: a review of neuroendocrine tumors and carcinomas. Neoplasia. 2017;19(12):991–1002. https://doi.org/10.1016/j.neo.2017.09.002
65 Pschowski R, Pape U-F, Fusch G, Fischer C, Jann H, Baur A, et al. Increased activity of the immunoregulatory enzyme indoleamine-2,3-dioxygenase with consecutive tryptophan depletion predicts death in patients with neuroendocrine neoplasia. Neuroendocrinology. 2017;104(2):135–44. https://doi.org/10.1159/000445191
66 Melhorn P, Mazal P, Wolff L, Kretschmer-Chott E, Raderer M, Kiesewetter B. From biology to clinical practice: antiproliferative effects of somatostatin analogs in neuroendocrine neoplasms. Ther Adv Med Oncol. 2024;16:17588359241240316. https://doi.org/10.1177/17588359241240316
67 Su X, Gao Y, Yang R. Gut microbiota-derived tryptophan metabolites maintain gut and systemic homeostasis. Cells. 2022;11(15):2296. https://doi.org/10.3390/cells11152296
68 Botticelli A, Cerbelli B, Lionetto L, Zizzari I, Salati M, Pisano A, et al. Can Ido activity predict primary resistance to anti-PD-1 treatment in NSCLC? J Transl Med. 2018;16(1):219. https://doi.org/10.1186/s12967-018-1595-3