IRIS AperTO

UNIVERSITÀ DEGLI STUDI DI TORINO

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Prognostic factors in ectopic Cushing's syndrome due to neuroendocrine tumors: A multicenter study

This is the author's manuscript

Original Citation:

Availability:

This version is available http://hdl.handle.net/2318/1634079 since 2019-04-26T11:39:07Z

Published version:

DOI:10.1530/EJE-16-0809

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

24 April 2024

IRIS AperTO

UNIVERSITÀ DEGLI STUDI DI TORINO

This is the author's final version of the contribution published as:

Davi' MV, Cosaro E, Piacentini S, Reimondo G, Albiger N, Arnaldi G, Faggiano A, Mantovani G, Fazio N, Piovesan A, Arvat E, Grimaldi F, Canu L, Mannelli M, Ambrogio AG, Pecori Giraldi F, Martini C, Lania A, Albertelli M, Ferone D, Zatelli MC, Campana D, Colao A, Scaroni C, **Terzolo M**, De Marinis L, Cingarlini S, Micciolo R, Francia G.

Prognostic factors in ectopic Cushing's syndrome due to neuroendocrine tumors: a multicenter study

European Journal of Endocrinology, 176(4); 2017; 451-459. DOI: 10.1530/EJE-16-0809

The publisher's version is available at:

[inserire URL sito editoriale presa dal campo URL, cioè dc.identifier.url]

http://www.eje-online.org/content/176/4/453.long

When citing, please refer to the published version.

Link to this full text:

[inserire l'handle completa, preceduta da http://hdl.handle.net/]

http://www.eje-online.org/content/176/4/453.full.pdf+html

This full text was downloaded from iris-Aperto: https://iris.unito.it/

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

1 Prognostic factors in ectopic Cushing's syndrome due to neuroendocrine tumors: a multicentre study 2

. 3 Maria Vittoria Davi'[1], Elisa Cosaro[1], Serena Piacentini[2], Giuseppe Reimondo[3], Nora Albiger[4], Giorgio

. 4 Arnaldi[5], Antongiulio Faggiano[6], Giovanna Mantovani[7], Nicola Fazio[8] , Alessandro Piovesan[9],

. 5 Emanuela Arvat[9], Franco Grimaldi[10] , Letizia Canu[11], Massimo Mannelli[11], Alberto Giacinto

. 6 Ambrogio[12 13], Francesca Pecori Giraldi[12 13] , Chiara Martini[14,] , Andrea Lania[15] , Manuela Albertelli[16],

. 7 Diego Ferone[17], Maria Chiara Zatelli[18], Davide Campana[19], Annamaria Colao[20], Carla Scaroni[4] ,

. 8 Massimo Terzolo[3], Laura De Marinis[2] , Sara Cingarlini[21], Rocco Micciolo[22], Giuseppe Francia[1].

[1*]Section of Endocrinology, Department of Medicine, University of Verona, [2*]Endocrinology Unit, Catholic University of Sacred Heart, Policlinico A. Gemelli, Rome, [3]Internal Medicine 1, Department of Clinical and Biological Sciences, University of Turin, San Luigi Gonzaga Hospital, Orbassano, [4]Endocrinology Unit, Department of Medicine DIMED, University Hospital, Padua, [5]Clinica di Endocrinologia e Malattie del Metabolismo Ospedali Riuniti di Ancona, Ancona, [6*]Thyroid and Parathyroid Surgery Unit, Istituto Nazionale per lo Studio e la Cura dei Tumori "Fondazione G. Pascale" IRCCS, Napoli; [7]Endocrinology and Diabetology Unit, University of Milan, Milan; [8*]Unit of Gastrointestinal Medical Oncology and Neuroendocrine Tumors, European Institute of Oncology, IEO, Milan; [9]Oncological Endocrinology Unit, Department of Medical Sciences, University of Turin, [10]Endocrinology, Diabetes, Metabolism and Clinical Nutrition Unit, University-Hospital S. Maria della Misericordia, Udine, [11]Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence, [12]Department of Clinical Sciences & Community Health, University of Milan, [13]Neuroendocrine Research Laboratory, Istituto Auxologico Italiano, Milan, Italy; [14]Internal Medicine, Department of Medicine, DIMED, University of Padova, [15*]Department of Biomedical Sciences,

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

Humanitas University and Endocrinology Unit, Humanitas Research Hospital, Rozzano, [16]Endocrinology, Department of Internal Medicine (DiMI), University of Genoa, [17]Endocrinology, Department of Internal Medicine (DiMI), IRCCS, AOU San Martino IST and Center of Excellence for Biomedical Research (CEBR), University of Genoa, [18]Sezione di Endocrinologia e Medicina Interna, Department of Medical Sciences, University of Ferrara, [19]Department of Medical and Surgical

1

eje@bioscientfica.com

For Review Only

Sciences, University of Bologna, [20]Endocrinology Division, Department of Clinical Medicine and Surgery, Università di Napoli Federico II, [21]Oncology, University of Verona, [22]Department of Psychology and Cognitive Sciences, University of Trento, Italy *ENETS Center of excellence

28
29
30
31
32
33
34
35
36
37
38
39

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59

Short Title: Ectopic ACTH Syndrome and neuroendocrine tumorsKey terms: Cushing's syndrome, ectopic ACTH, neuroendocrine tumors, prognosis, treatment Word count of full article: 5034Corresponding author:

Maria Vittoria Davi' MD

Section of Endocrinology, Medicina Generale e Malattie Aterotrombotiche e Degenerative,

ENETS Center of excellence Department of Medicine, University of Verona Piazzale LA Scuro Policlinico G.B. Rossi 37134 Verona, Italy

Phone: +39-045-8124684Fax: +39-045-8027496 mariavittoria.davi@ospedaleuniverona.it

For Review

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

Only

. 60 ABSTRACT

. 61 Objective: evidence is limited regarding outcome of patients with ectopic Cushing's syndrome (ECS) due to

. 62 neuroendocrine tumors (NETs).

. 63 Design: we assessed prognostic factors affecting survival of patients with NETs and ECS.

. 64 Methods: retrospective analysis of clinicopathological features, severity of hormonal syndrome, treatments

. 65 from a large cohort of patients with NETs and ECS collected from 17 Italian centres.

. 66 Results: our series included 110 patients, 58.2% female, with mean (±SD) age at diagnosis of 49.5 ±15.9

. 67 years. The main sources of ectopic ACTH were: bronchial carcinoids (BC) (40.9%), occult tumors (22.7%)

. 68 and pancreatic (p)NETs (15.5%). Curative surgery was performed in 56.7% (70.2% of BC, 11% of pNETs).

. 69 Overall survival was significantly higher in BC compared with pNETs and occult tumors (p=0.033) and in

. 70 G1-NETs compared with G2 and G3 (p=0.007). Negative predictive factors for survival were severity of

. 71 hypercortisolism (p<0.02), hypokalemia (p=0.001), diabetes mellitus (p=0.0146) and distant metastases

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

. 72 (p<0.001). Improved survival was observed in patients who underwent NET removal (p<0.001).

. 73 Adrenalectomy improved short-term survival.

. 74 Conclusions: multiple factors affect prognosis of ECS patients: type of NET, grading, distant metastases,

. 75 severity of hypercortisolism, hypokalemia and diabetes mellitus. BC have the highest curative surgical rate

. 76 and better survival compared with occult tumors and pNETs. Hypercortisolism plays a primary role in

. 77 affecting outcome and quality of life; therefore, prompt and vigorous treatment of hormonal excess by NET

. 78 surgery and medical therapy should be a key therapeutic goal. In refractory cases, adrenalectomy should be

. 79 considered, since it affects outcome positively at least in the first 2 years.

3

For Review Only

81
82
83
84

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105

Introduction

Ectopic Cushing's syndrome (ECS) is a rare condition accounting for 10-20% of all cases of ACTH- dependent Cushing (1,2). Neuroendocrine tumors (NETs), mainly bronchial carcinoids (BC) (3-54.8%) are the most frequent causes of ectopic ACTH secretion in more recent series (3-8), while small cell lung carcinoma (SCLC) represented the most common tumor associated with ECS in early series (3.3-50%) (9). Other less frequent causes are thymic carcinoids (TC) (5-42%), pancreatic (p) NETs (7.5-25%), pheochromocytomas (2.5-25%) and medullary thyroid carcinomas (MTC) (2-8%) (3-8). Unknown primary tumors account for 12-36.5% of all causes of ectopic ACTH production (3).

Available data on patients affected by ECS deriving from NETs is relatively scarce and outdated. The largest retrospective series is from the Mayo Clinic, including 106 patients followed between 1958 and 1986 (5). This study addressed the clinical characteristics, modalities of treatment and follow up of the patients, without reporting on histopathological features and prognosis of the tumors. In the second most numerous retrospective study, data regarding performance of diagnostic tests, therapies, pathological examinations and survival of 90 patients from NIH between 1983 and 2004 were analysed (6). It was found that 47% of the whole group, represented mainly by BC, underwent curative resection compared with 12% of the series from Mayo Clinic; patients affected by gastrinoma, MTC and SCLC displayed the poorest prognosis. In another series from UK including 40 patients, those affected by BC had the highest surgical curative rate (83%), whereas the remainders needed

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

adrenolytic therapy or adrenalectomy to control hypercortisolism (7). Histology and distant metastases were the main prognostic factors.

The aim of our study was to assess which factors affect survival on a large multicentre series of patients with NETs and ECS with complete clinical annotations.Patients and methodsWe did a retrospective analysis of data from patients with ECS from NETs collected in 17 Italian referral centres between 1986 and 2014, obtained by means of a specific questionnaire divided in different items. The study received the approval of the institutional ethical committees of each centre.

eje@bioscientfica.com

For Review Only

. 106 Patients with diagnosis of ECS due to NETs were identified from the institutional database of patients of

. 107 each centre. Data were retrieved by medical personnel from medical records both in paper and electronic

. 108 .

. 109 The diagnosis of ECS was based on a review of the patient's medical history, clinical features associated

. 110 with cortisol excess and laboratory tests. These included increase in 24-h urinary free cortisol (UFC) levels

. 111 in at least 2 samples, unsuppressed serum cortisol after 1-mg overnight dexamethasone suppression test

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

. 112 (DST), loss of physiological cortisol diurnal rhythm with assessment of midnight plasma and/or salivary

. 113 cortisol levels and increase in ACTH levels. To differentiate between pituitary and ectopic ACTH production

. 114 at least 2 of the following tests were performed: overnight 2-mg, 8-mg DST, 100-mcg CRH test and

. 115 desmopressin test.

. 116 The first item of the questionnaire referred to the site of ACTH production, histological features of NET

. 117 according to the 2010 WHO classification for gastroenteropancreatic (GEP) NETs (10) and to the 2004

. 118 WHO classification for lung carcinoids when applicable (11), proliferative activity by staining for the Ki-67

. 119 antigen or by mitotic count per 10 high–power fields, presence of local and distant metastases and

. 120 association with inherited syndrome, such as multiple endocrine neoplasia (MEN) type 1, MEN 2 and von

. 121 Hippel Lindau disease (VHL). Results from biopsy of the primary tumor or metastases were also reported

. 122 when available.

. 123 Diagnosis of occult/unknown primary tumor was reserved for patients with clinical features of

. 124 hypercortisolism and hormonal tests suggestive of an ectopic ACTH source including lack of central-

. 125 peripheral ACTH gradient during inferior petrosal sinus sampling (IPSS), without primary tumor localization

. 126 after prolonged and repeated imaging follow-up established by negative radiological and/or nuclear medicine

. 127 procedures, and exclusion of other tumors known to cause ECS. In 2 cases of occult ECS of our series

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

128

. 129 Other items regarded clinical data, including type and duration of symptoms before ECS diagnosis, the

. 130 biochemical and hormonal profile including serum potassium, 24-h UFC, serum cortisol at 8 a.m., at

. 131 midnight and salivary if available, plasma ACTH, dynamic tests [overnight 1-mg, 2-mg, 8-mg DST, 100

. 132 mcg CRH test, desmopressin test and central-peripheral gradients of baseline and CRH stimulated ACTH 5

form.	The centre
pituitary esploration had been performed without the finding of any pituitary lesion	(12).

eje@bioscientfica.com

For Review Only

133
134
135
136
137
138
139
140
141
142

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

assessed during IPSS. Considering different assay techniques, hormonal levels were classified in 3 categories; 1) up to 3-fold increase over the upper limit of normal (ULN) of the reference range of each centre, 2) between 3- to 5-fold increase, and 3) above 5-fold increase. Chromogranin A and neuron specific enolase (NSE) levels were also collected when available. Other specific items were pituitary magnetic resonance imaging (MRI) and diagnostic imaging procedures performed to identify the NET, including radiological an nuclear medicine ones. Data regarding modalities of treatments for ECS (steroidogenesis inhibitors, adrenalectomy) and for tumor control [surgery of the NET, antiproliferative agents, transcatheter arterial chemoembolization (TACE), transcatheter arterial embolization (TAE), radiofrequency ablation (RFA), selective internal radiation therapy (SIRT), peptide receptor radionuclide therapy (PRRT)] and results of treatment in terms of ECS control (complete or partial defined as UFC level above the ULN, but reduced by ≥ 50% from baseline) and survival were collected.

Statistical analysis

The results are expressed as means (±SD) or median. Two-sample Student's t-test was used to verify statistical differences between means while chi square test to evaluate the association between categorical variables. Values of $p<0.05$ were considered statistically significant. Survival probabilities were estimated employing the Kaplan-Meier method. Survival curves were compared employing the log-rank test. The joint prognostic role of considered variables was evaluated employing the Cox model. All the analyses were performed employing the software R (R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing (Vienna, Austria).

Results

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

Clinicopathological characteristics of the patients

The study included 110 patients (58.2% female) diagnosed with ECS from NET, with mean (±SD) age at

diagnosis of 49.5 ±15.9 years. The clinicopathological characteristics of the patients are summarized in Table 1. The sources of ectopic ACTH were 45 (40.9%) BC (30 typical, 12 atypical and 3 not otherwise specified), 25 (22.7%) occult/unknown primary tumors, 17 (15.5%) pNETs, 7 (6.4%) pheochromocytomas, 6 (5.5%) TC, 4 (3.6%) SCLC, 3 (2.7%) intestinal (1 ileal, 1 caecal, 1 rectal) NETs, 2 (1.8%) MTC and 1 (0.9%) small cell carcinoma of the cervix (SCCC).

eje@bioscientfica.com

For Review Only

. 160 Ki 67% or mitotic count was available in 54 NETs, allowing stratification in 3 groups: 1) G1 NETs

. 161 characterized by Ki 67%/mitotic count of 0-2, including 17 typical BC, 3 pNETs, 1 pheochromocytoma

. 162 (38.9% of patients); 2) G2 NETs characterized by Ki 67%/mitotic count of 3-20, including 9 atypical BC, 6

. 163 pNET, 3 TC, 1 pheochromocytoma, 1 caecal, 1 metastatic occult tumor; (38.9% of patients); 3) G3 NETs

. 164 characterized by Ki 67%/mitotic count of >20 including 4 pNETs, 2 atypical BC, 2 metastatic occult tumors,

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

. 165 1 SCLC, 1 TC, 1 rectal NET, 1 SCCC (22.2% of patients). Immunostaining for ACTH was reported positive

. 166 in 42/52 (84.6%)

. 167 Distant metastases were significantly (chi square=40.9; p<0.001) more prevalent in p-NETs (13/17, 76.5%),

. 168 SCLC (3/4, 75%), TC (5/6 83%) and atypical BC (6/12 50%) than in typical BC (4/30 13.3%) and

. 169 metastases form occult tumors (4/25 16%). No pheocromocytoma was malignant. One out of 2 MTC had

. 170 distant metastases. Lymph node metastases were present in 11/30 (36.7%) typical BC, 8/12 (66.7%) atypical

. 171 BC, 8/17 (47%) pNETs, 3/6 (50%) TC, 2/25 (8%) occult tumors, 2/4 (50%) SCLC, 2/2 MTC (100%). These

. 172 percentages were significantly different (chi square=22.7; p=0.004).

. 173 Clinical features at diagnosis are summarized in Table 2. No significant difference neither in the prevalence

. 174 of symptoms nor in the number of symptoms was found among the type of NET subgroups

. 175 The mean duration of symptoms before NET diagnosis was 13.1 ± 21.2 months.

. 176 Two (1.8%) patients (1 with pNET and 1 with typical BC) were affected by MEN1 and 1 (0.9%) with pNET

. 177 by Von Hippel Lindau disease.

. 178 Laboratory data

. 179 Hypokalemia (serum potassium <3.5 mEq/l) was present in 72 (71.3%) patients. 24-hr UFC was increased in

. 180 91/93 (97.8%) patients, s-cortisol at 8 a.m. in 87/105 (82.9%) and ACTH in 97/102 (95.1%). In the 2 patients

. 181 with normal 24-hr UFC the diagnosis of ECS was made on the basis of at least other 2 altered tests. Serum

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

. 182 cortisol at midnight was collected in 52/110 (47.3%) patients and was increased in all. No significant

. 183 difference was observed in hormonal levels among the subgroups of NETs. A significant correlation was

. 184 found between hormonal levels (s-cortisol, 24-hr UFC, ACTH) and hypokaliemia ($p<0.05$), but not between

. 185 hormonal levels and clinical features. The laboratory data and the sensitivity of dynamic tests are

. 186 summarized in Table 3. 7

eje@bioscientfica.com

For Review Only

187
188
189
190
191
192
193
194
195
196
197
198
199

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

Diagnostic proceduresIn all cases (45) in which pituitary MRI was carried out no pituitary mass was found.With regard to occult tumors, 21/25 patients had undergone computed tomography (CT), 8/25 patients MRI, 17/25, somatostatin-receptor scintigraphy (SRS), 6/25 ^{18}F-FDG-PET/CT, 4/25 ^{68}Ga-DOTA-peptide PET/CT, 17/25 radiological imaging (CT/MRI) plus a functional imaging (SRS/PET), 1 colonoscopy. In 4 cases (3 typical BC and 1 TC), the NET was found after a mean time of 4 years (range 3-5 years) from the ECS diagnosis by CT scan in all, ^{68}Ga-DOTA-peptide PET/CT in 2 and SRS in 1. Two of them had been submitted to adrenalectomy as first therapeutic choice.The prevalence of occult tumors among patients recruited in the period between 2006 and 2014 decreased (17.2%), compared with those recruited in the previous periods (25% between 1986 and 1994, and 32.4% between 1995 and 2005).Medical treatment of ECSThe majority of patients (78/110, 70.9%) were treated with steroidogenesis inhibitors, even in combination [ketoconazole in 69/78 (88.5%), mitotane in 13/78 (16.7%) and metyrapone in 5/78 (6.4%)]. Other drugs were the glucocorticoid receptor antagonist mifepristone in 1 (0.9%), cabergoline in 8 (7.3%) and somatostatin analogues (SA: lanreotide or octreotide LAR) in 60 (54.5%). In 59/110 (53.6%) patients each drug was given alone, whereas in 51/110 (46.4%) in combination, the most used being ketoconazole plus SA (41 patients) or mitotane plus SA (11 patients). Steroidogenesis inhibitors were used before surgery in 33/60 patients (55%), and after surgery in 4/60 (6.6 %). Complete hormonal control was achieved in 15/51 (29.4%) and partial control in 36/51 (70.6%) patients who received only medical treatment.

Surgical treatment of the NET and adrenalectomy

Sixty out of 110 (54.5%) patients underwent etiologic surgery: 37/45 (82.2%) BC (8 atypical, 26 typical, 3 unspecified), 9/17 (52.9%) pNET, 5/6 (83.3%) TC, 5/7 (71.4%) pheocromocytomas, 1 SCCC, 1 ileal NET, 1 MTC and 1 patient who resulted a false positive. Curative surgery was obtained in 34/60 (56.7%): 26/37 (70.2%) BC (21 typical, 4 atypical, 1 not otherwise specified), 1/9 (11.1%) pNETs , 5/5 pheocromocytomas (100%), 1 TC, 1 ileal NET. In 8 ECS relapsed (4 typical BC, 1 atypical BC, 2 BC not otherwise specified, 1 pNET), after a mean time of 5.2 years from surgery. Palliative surgery, i.e.

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

resection of the primary in the

eje@bioscientfica.com

For Review Only

. 214 presence of distant metastases was performed in 16/60 patients (26.6%), of whom 1 typical BC, 3 atypical

. 215 BC, 4 TC, 7 pNETs, and 1 SCCC.

. 216 Thirty-one (28.2%) patients underwent adrenalectomy: 18 as primary treatment (5 BC, 1 TC, 4 pNETs, 8

. 217 occult tumors), and 13 following other treatments (4 BC, 3 pNETs, 2 TC, 4 occult tumors). Five out of 31

. 218 patients (2 pNETs, 1 BC, 1 TC, 1 occult tumor) underwent monolateral adrenalectomy due to poor clinical

. 219 conditions with the intent of 2-step adrenalectomy. The prevalence of patients who underwent adrenalectomy

. 220 was significantly higher in occult tumors and p-NETs compared with BC (48% and 41.2% vs 20%, p=

. 221 0.038).

. 222 Antineoplastic treatment

. 223 As additional therapy, 8 (7.3%) patients (1 BC, 1 TC, 5 pNETs, 1 occult tumor)

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

received Everolimus and 1

. 224 (0.9%) patient (pNET) Sunitinib. Chemotherapy was performed in 27 (24.5%) patients including

. 225 strepto/doxo/5FU in 3 patients (1 atypical BC, 1 TC, 1 pNET), etoposide/cisplatin in 14 patients (2 atypical

. 226 BC, 2 SCLC, 3 TC, 4 pNETs, 1 SCCC, 2 occult tumors) and temozolomide/capecitabine 10 patients (1

. 227 typical BC, 1 atypical BC, 1 TC, 5 pNETs, 1 occult tumor, 1 caecal NET). Chemotherapy was administered

. 228 alone in 12 (44.4%) patients, in combination with surgery of the NET in 12 patients and after tumor relapse

. 229 in 3 (11.1%) patients.

. 230 TACE/TAE of liver metastases were performed in 5 (4.5%, all pNETs) patients, RFA in 3 (2.7%) of whom 2

. 231 pNET, 1 BC, SIRT in 1 (0.9%) BC. PRRT was carried out in 13 (11.8%) (6 BC, 2 TC, 5 pNETs) of whom

. 232 10 (76.9%) obtained a partial or complete ECS control.

. 233 Follow up and survival rate

. 234 Considering the whole population of 110 patients the median follow up (from diagnosis to last evaluation)

. 235 was 60 months. During the follow-up period, 30 patients died: 18 patients (2 typical BC, 2 atypical BC, 2

. 236 TC, 5 pNETs, 1 caecal, 1 SCCC, 1 MTC, 4 occult tumors) due to tumor progression, 11 patients (2 typical

. 237 BC, 2 SCLC, 2 pNETs, 1 rectal, 4 occult tumors) due to the consequences of cortisol excess (4 respiratory

. 238 failure secondary to thromboembolism or pneumonia, 1 acute myocardial infarction, 5 septic shock, 1 severe

. 239 hypokalemia) and 1 patient with occult tumor died for breast adenocarcinoma. 9

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

For Review Only

240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

The overall survival (OS) was: 84% at 1 year of follow up, 81% at 2 years, 74% at 3 years, 70% at 4 and 5 years.Patients ≥60-yr-old at diagnosis had a worse prognosis than younger patients (5-yr OS 52% vs 81% <40 yr- old and vs 77% between 40-59 yrs old at diagnosis, p=0.016).

There was no statistically significant difference in OS according to gender and period of diagnosis.The 5-yr OS rate for BC was significantly higher than that for pNETs and occult tumors (86% vs 60% for latter groups, p=0.033) (Figure 1). With regard to the histologic grade, there was a significant trend toward worsening of 5-yr OS for G1, G2 and G3-NETs (87.7%, 72.4%, 37.0% respectively; p=0.007).Patients with metastases had a poorer prognosis than those without (5-yr OS: 79% vs 61%, p=0.019). This was true in particular for patients with distant metastases (5-yr OS: 82% vs 47%, p<0.001) (Figure 2). Considering the s-cortisol levels a worse survival rate was found in the group with >3-fold increase compared with the groups with up to 3-fold increase (p<0.001). Similarly for 24-hr UFC and ACTH, patients with > 5-fold increase had a worse survival rate compared with patients with up to 5-fold increase (p=0.005 and p=0.01, respectively) (Figure 3). The presence of hypokalemia (<3.5 mEq/L) (p=0.001) and diabetes mellitus (p=0.0146) were negative prognostic factors.Patients who underwent etiologic surgery had a better survival rate than those who did not (5-yr OS 85% vs 51%, p<0.001) (Figure 4). Patients who underwent adrenalectomy had a better survival rate in the first 2 years compared with those who did not. During the first 2 years of follow-up there were no deaths in the former group vs 18 deaths in the latter, with a 2-year overall survival of 74%, although the 5-yr OS was not significantly different (76% vs 68%, p=0.168).The Cox model was employed to evaluate the joint effect of the following variables: age at diagnosis, distant metastases, NET surgery, adrenalectomy, Ki-67 and 24-hr UFC. A significance prognostic role (p<0.001) was found for distant metastases and NET surgery. For the first variable the hazard ratio (yes vs no) was 5.4 (95% C.I.: 2.2-13.3), while for the second (no vs yes) was 6.1 (95% C.I.: 2.4-15.7). Also the ≥5-fold increase over ULN of 24-hr UFC was significantly associated with worse prognosis (p=0.012); the estimate of the hazard ratio was 6.5 (95% C.I.: 1.5-28.2).

eje@bioscientfica.com

For Review Only

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

. 267 Discussion

. 268 The aim of our study was to assess the factors affecting survival in patients with ECS due to NETs. The

. 269 strengths of our study are the sample size, the largest up to now reported, and the complete clinical

. 270 annotation of our patients allowing assessment of factors never previously examined on a large scale. We

. 271 acknowledge the limitation of the retrospective and multicentre nature of the study.

. 272 The major finding of our study is that the severity of cortisol excess impacts on survival. Hypercortisolism

. 273 causes severe comorbidities due to its effects on glucose and lipid metabolism, the cardiovascular system,

. 274 skeletal turnover and the immune system (13-16). It is known that the consequences of hypercortisolism can

. 275 lead to the death of the patient, sometimes more rapidly than tumor progression (17, 18). However, until now

. 276 the correlation between cortisol excess and survival had not yet been demonstrated on a large scale. We have

. 277 herein shown that the severity of cortisol excess significantly affects overall survival and that the increase in

. 278 24-hr UFC is an independent negative prognostic factor. This is a key clinical message for an appropriate

. 279 management of ECS, prompting that a vigorous control of the hormonal syndrome should be one of the main

. 280 therapeutic goals. Our results show that the outcome of patients with ECS did not improve over the 30 years

. 281 of the study. As hypercortisolism is among the major causes of a poor prognosis in

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

these patients, it seems

. 282 advisable to actively counteract it with a more rapid and effective medical therapy or, in case of failure, an

. 283 early bilateral adrenalectomy.

. 284 Our study also demonstrates how tumor grading and staging affect the prognosis of these patients. This is the

. 285 first study to demonstrate that survival is progressively and significantly improved for moderate and well

. 286 differentiated NETs compared with that for poorly differentiated NETs. Overall survival was significantly

. 287 worse for patients with distant metastases, as already shown in other studies (7,8).

. 288 Considering the site of the primary tumor, BC were confirmed to be the most prevalent source of ectopic

. 289 ACTH, as reported in the most recent series (5-8). Typical BC were more frequent than atypical BC (66% vs

. 290 26.6%) and showed a more favourable behaviour with less lymph node and distant spread (36.7% vs 66.7%

. 291 and 13% vs 50% respectively). Surgery was curative in a high proportion of patients with BC (70.2%) even

. 292 in the presence of positive lymph nodes without the need of post-operative mediastinal radiotherapy. 11

For Review Only

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319

Moreover, BC showed a significantly better survival rate compared with that of pNETs and occult tumors. The favourable survival rate of BC compared with other ACTH-secreting tumors had already been observed in other studies (6, 7, 19). Conversely, survival rate of occult tumors in our series was poorer than that of BC and similar to that of pNET, in contrast to the results reported by Ilias et al. (6). Pancreatic NETs were the most frequent source of ECS among GEP-NETs; they were usually of great size with distant metastases. The majority of pNETs (9/13) were well differentiated (G2 and G1) as found in a recent report (20). These tumors did not have distinctive histological features compared with pNETs without ECS; however, they displayed signs of aggressiveness including vascular and perineural invasion. In our study the outcome of pNETs was worse compared with that of BC, with a lower curative surgical rate (11% vs 70.2%) and a lower 5-yr OS (60% vs

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

86%).

A significant percentage of tumors in our series (22.7%) remained occult after diagnostic work-up.

There is a current debate on the possibility of the presence of Cushing's disease in the patient with negative IPSS without evidence of ectopic tumor (21). In only 2 cases of our series pituitary exploration had been performed without the finding of any pituitary lesion. The majority of our centres did not routinely perform surgical exploration of the pituitary in presence of concordant dynamic tests for ECS and negative IPSS. Only 4/25 patients had undergone ^{68}Ga-DOTA-peptide PET/CT, which is known to be highly sensitive in detecting NETs due to the limited and only recent availability among the centres. Some BC may be too small to be identified by CT scan or show heterogeneity of SSTR expression preventing them from being visualized by SRS (22). It is worth noting that in some cases the negative somatostatin receptor scintigraphy or ^{68}Ga-DOTA-peptide PET/CT can be due to down-regulated SSTR expression after long-term exposure to hypercortisolism (23,24).

The survival rate of patients with occult tumors and ECS was similar to that of patients with pNETs but worse than that of patients with BC due to the metastatic spread or the consequences of hypercortisolism. Only one third of the patients (8/25) with occult tumors underwent adrenalectomy as first treatment. In our series, patients with a higher increase in hormonal levels or a presence of hypokalemia and diabetes mellitus, and those who did not perform surgery of the NET had a worse prognosis. Furthermore, patients who underwent adrenalectomy had a better survival rate at least in the first 2 years, highlighting the importance of

eje@bioscientfica.com

For Review Only

. 320 prompt control of hypercortisolism. Nowadays, laparoscopic adrenalectomy is a safe

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

and minimally invasive

. 321 surgical treatment (25); thus, it should be considered more frequently as an option in the management of

. 322 ECS, in particular in early stages, when the hormonal syndrome is not controlled by medical treatment

. 323 and/or persists after surgical removal of the primary tumor.

. 324 Concerning the outcome of medical therapy, the variability of treatments employed did not allow us to draw

. 325 a definitive conclusion.

. 326 In our experience, PRRT was an effective therapeutic option when SRS or ^{68}Ga-DOTA-peptide-PET/CT are

. 327 positive, attaining partial or complete hormonal control in 76.9% of patients.

. 328 Conclusions

. 329 Several factors have an impact on prognosis of patients with ECS, namely, NET type, grading, presence of

. 330 distant metastases, severity of hypercortisolism, hypokalemia and diabetes mellitus. BC have the highest

. 331 curative surgical rate and better survival than occult tumors, which still account for 23% of cases, and

. 332 pNETs. Cortisol excess plays a major role in affecting duration and quality of life; therefore, a prompt

. 333 treatment directed toward the primary tumor and inhibiting adrenal function is a key therapeutic goal. In

. 334 refractory cases, adrenalectomy should be considered, since it affected positively patients' outcome at least

. 335 in the first 2 years.

336

. 337 Declaration of interest: MVD, EC, SP, NA, GM, AP, EA, FG, LC, MM, AGA, FPG,

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

CM, AL, MA, DF,

. 338 MCZ, DC, CS, LDM, SC, RM, GF have nothing to disclose.

. 339 GR received research grants from Novartis and Shire; GA received research grants from Novartis and Shire;

. 340 speaker fees from Novartis, consulting fees from Novartis and Pfizer, Inc. as a board Member; AF received

. 341 speaker fees from Novartis and Ipsen; NF is a member of Novartis and Ipsen Advisory Board; AC received

. 342 research grants from Novartis, received speaker fees from Novartis and Ipsen, is a member of Novartis and

. 343 Ipsen Advisory Board; MT is a member of HRA Pharma Advisory Board and received HRA Pharma

. 344 research grant.

. 345 Funding: This research did not receive any specific grant from any funding agency in the public,

. 346 commercial or not-for-profit sector. 13

eje@bioscientfica.com

For Review Only

347
348

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

349
350
351
352
353 354 355

356 357

358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
References

1. Wajchenberg BL, Mendonca BB, Liberman B, Pereira MA, Carneiro PC, Wakamatsu A, Kirschner MA. Ectopic adrenocorticotropic hormone syndrome. Endocrine Reviews 1994 15 752-8.2. Newell-Price J, Bertagna X, Grossman AB, Nieman LK. Cushing's syndrome. Lancet 2006 367 1605–17 3. Alexandraki KI, Grossman AB. The ectopic ACTH syndrome. Reviews in Endocrine and Metabolic Disorders 2010 11 117-126.

4. Isidori AM, Sbardella E, Zatelli MC, Boschetti M, Vitale G, Colao A, Pivonello R; ABC Study Group. Conventional and Nuclear Medicine Imaging in Ectopic Cushing's Syndrome: A Systematic Review. The Journal of Clinical Endocrinology & Metabolism 2015 100 3231-44.

5. Aniszewski JP, Young WF Jr, Thompson GB, Grant CS, van Heerden JA. Cushing syndrome due to ectopic adrenocorticotropic hormone secretion. World Journal of Surgery 2001 25 934-40.

6. Ilias I, Torpy DJ, Pacak K, Mullen N, Wesley RA, Nieman LK. Cushing's syndrome due to ectopic corticotropin secretion: twenty years' experience at the National Institutes of Health. Journal of Clinical Endocrinology & Metabolism 2005 90 4955-62.7. Isidori AM, Kaltsas GA, Pozza C, Frajese V, Newell-Price J, Reznek RH, Jenkins PJ, Grossman AB,

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

Besser GM. The ectopic adrenocorticotrophin syndrome: clinical features, diagnosis, management and long- term follow-up. Journal of Clinical Endocrinology & Metabolism 2006 91 371-7.

8. Kamp K, Alwani RA, Korpershoek E, Franssen GJ, de Herder WW, Feelders RA. Prevalence and clinical features of the ectopic ACTH syndrome in atients with gastroenteropancreatic and thoracic neuroendocrine tumors. European Journal of Endocrinology 2016 174 271-80.9. Shepherd FA, Laskey J, Evans WK, Goss PE, Johansen E, Khamsi F. Cushing's syndrome associated with ectopic corticotropin production and small-cell lung cancer. Journal of Clinical Oncology 1992 10 21-27.

10. Klöppel G, Perren A, Heitz PU. The gastroenteropancreatic neuroendocrine cell system and its tumors: the WHO classification. Annals of the New York Academy of Sciences 2004 1014 13-27.11. Travis W, Brambilla E, Muller-Hermelink H, Curtis CH. Tumours of the lung, pleura, thymus and heart. Lyon: IARC Press 2004.

eje@bioscientfica.com

For Review Only

. 373 12. Isidori AM, Kaltsas GA, Grossman AB. Ectopic ACTH syndrome. Frontiers of Hormone Research 2006

. 374 35 143-56.

375 376

377
378
379
380

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

. 381 Torpy DJ, Mullen N, Ilias I, Nieman Association of hypertension and hypokalemia with Cushing's

. 382 syndrome caused by ectopic ACTH secretion: a series of 58 cases. Annals of the New York Academy of

. 383 Sciences 2002 970 134-44.

384
385
386
387
388
389
390
391

. 392 Maragliano R, Vanoli A, Albarello L, Milione M, Basturk O, Klimstra DS, Wachtel A, Uccella S, Vicari

. 393 E, Milesi M, et al. ACTH-secreting pancreatic neoplasms associated with Cushing syndrome:

. 394 clinicopathologic study of 11 cases and review of the literature. The American Journal of Surgical Pathology

. 395 2015 39 374-82.

. 396 21.Sheth SA, Mian MK, Neal J, Tritos NA, Nachtigall L, Klibanski A, Biller BM, Swearingen B.

. 397 Transsphenoidal surgery for cushing disease after non diagnostic inferior petrosal sinus sampling.

. 398 Neurosurgery 2012 71 14-22. 15

13

. Isidori AM, Graziadio C, Paragliola RM, Cozzolino A, Ambrogio AG, Colao A, Corsello SM, Pivonello R; ABC Study Group The hypertension of Cushing's syndrome: controversies in the pathophysiology and focus on cardiovascular complications.. Journal of Hypertension 2015 33 44-60.14. Isidori AM, Minnetti M, Sbardella E, Graziadio C, Grossman AB. Mechanisms in endocrinology: The spectrum of haemostatic abnormalities in glucocorticoid excess and defect. European Journal of Endocrinology 2015 173 101-13.

15.

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

16

. Pivonello R, Isidori AM, De Martino MC, Newell-Price J, Biller BM, Colao A. Complications of Cushing's syndrome: state of the art. The Lancet Diabetes & Endocrinology 2016 4 611-29.17. Clayton RN. Mortality in Cushing's Disease. Neuroendocrinology 2010 92 71-76.18. Steffensen C, Bak AM, Rubeck KZ, Jørgensen JO. Epidemiology of Cushing's Syndrome. Neuroendocrinology 2010 92 1-5.

19. Salgado LR, Fragoso MC, Knoepfelmacher M, Machado MC, Domenice S, Pereira MA, de Mendonça BB. Ectopic ACTH syndrome: our experience with 25 cases. European Journal of Endocrinology 2006 155 725-33.

20.

eje@bioscientfica.com

For Review Only

22.

Anaforoğlu I, Ersoy K, Aşık M, Karyağar S, Algün E. Diagnosis of an ectopic adrenocorticotropic hormone secreting bronchial carcinoid by somatostatin receptor scintigraphy. Clinics (Sao Paulo) 2012 67 973-5.

23.

399
400
401
402
403
404
405

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

406 407 408

409
410
411
412
de Bruin C, Hofland LJ, Nieman LK, van Koetsveld PM, Waaijers AM, Sprij-Mooij DM, van Essen M, Lamberts SW, de Herder WW, Feelders RA. Mifepristone Effects on Tumor Somatostatin Receptor Expression in Two Patients with Cushing's Syndrome due to Ectopic Adrenocorticotropin Secretion. Journal of Clinical Endocrinology & Metabolism 2012 97 455-462.

Davi' MV, Salgarello M, Francia G. Positive (68)Ga-DOTATOC-PET/CT after cortisol level control during ketoconazole treatment in a patient with liver metastases from a pancreatic neuroendocrine tumor and ectopic Cushing syndrome. Endocrine 2015 49 566-7.

Reincke M, Ritzel K, Oßwald A, Berr C, Stalla G, Hallfeldt K, Reisch N, Schopohl J, Beuschlein F. A critical reappraisal of bilateral adrenalectomy for ACTH-dependent Cushing's syndrome. European Journal of Endocrinology 2015 173 23-32.

24.

25.

eje@bioscientfica.com

For Review Only

. 413 Figure legends:

. 414 Figure 1. Overall survival by neuroendocrine tumor (NET). BC: bronchial carcinoid; pNET, pancreatic

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

. 415 NET, occult tumor. Number of patients at risk at 12, 24, 36, 48, 60 months are 36, 32, 27, 24, 21 (BC), 11,

. 416 10, 8, 6, 4 (pNET), 18, 16, 13, 10, 10 (Occult tumor). Survival probabilities were estimated employing the

. 417 Kaplan-Meier method. Survival curves were compared employing the log-rank test.

. 418 Figure 2. Overall survival by distant metastases. Number of patients at risk at 12, 24, 36, 48, 60 months are

. 419 21, 17, 10, 9, 7 (Yes), 57, 52, 45, 37, 31(No). Survival probabilities were estimated employing the Kaplan-

. 420 Meier method. Survival curves were compared employing the log-rank test.

. 421 Figure 3. Overall survival by 24-hr UFC levels. UFC: urinary free cortisol; ULN: upper limit of normal.

. 422 Number of patients at risk at 12, 24, 36, 48, 60 months are 23, 20, 16, 14, 11 (up to 5 ULN), 40, 36, 32, 26,

. 423 21 (> 5 ULN). Survival probabilities were estimated employing the Kaplan-Meier method. Survival curves

. 424 were compared employing the log-rank test.

. 425 Figure 4. Overall survival by neuroendocrine tumor (NET) surgery. Number of patients at risk at 12, 24, 36,

. 426 48, 60 months are 49, 46, 37, 33, 26 (Yes), 29, 23, 18, 13,12 (No). Survival probabilities were estimated

. 427 employing the Kaplan-Meier method. Survival curves were compared employing the log-rank test.

eje@bioscientfica.com

For Review

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

Only

Table 1. Clinicopathological characteristics of the patients

BCOccult tumors	pNETs	Pheochromocytomas	TCSCLC	Intestinal NETs	MTCSCCC					
Source of ectopic ACTH - no. (%)										
45 (40.9)	25 (22.7)	17 (15.5)	7 (6.4)							
6 (5.5)	4 (3.6)	3 (2.7)	2 (1.8)	1 (0.9)						
Mean ± SD age at diagnosis (yrs)										
44.9 ± 16.6	55.4 ± 13.5*	48.3 ± 14.7	50.1 ±17	42±14						
69.7 ± 7.6*	66±9	57.5 ± 3.5	24							
Mean ± SD tumor diameter										
(mm) 23.4 ± 18										
-48.4 ± 34.7*	26.5 ± 14	57.4 ± 9.9*	33.3 ± 5.8	21.5 ± 13.4	3042					
Mean ± SD symptom duration before diagnosis (months)										
16.5 ± 24.5	18.5 ±28.3	3.9 ± 5.6*	11 ± 8.5	11.6 ± 8.3	3.25 ± 2.1*	13 ± 19.92	6.5			
5										
Distant metastases - no./total no. (%)										
9/36 (20)	4/25 (16)	13/17 (76)*	0/7 (0)	5/6(83)*	3/4 (75)*	2/3 (66.7)	1/2 (50)	1/1 (100)		
Lymph node metastases - no./total no. (%)										
20/45 (44.4)*	2/25 (8)	8/17(52.9)*	0/7 (0)							
3/6 (50)*	2/4 (50)*	1/3 (33.3)	2/2 (100)	1/1 (100)						

*p < 0.05; NETs, neuroendocrine tumors; BC, bronchial carcinoid; pNETs, pancreatic neuroendocrine tumors; TC, thymic carcinoids; SCLC, small cells lung cancer; MTC, medullary thyroid carcinoma; SCCC, small cells

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

cervical carcinoma; SD, standard deviation.

For Review Only

Table 2. Clinical features at diagnosis Clinical features at diagnosis %

Hypertension 89.1 Diabetes mellitus 65.5 Proximal myopathy 70.9 Skin fragility 54.5 Osteoporosis 48.2 Psychiatric diseases 34.5 Hypercoagulability 30 Serum potassium <3.5 mEq/L 71.3

eje@bioscientfica.com

For Review Only

Table 3. Laboratory data and sensitivity of dynamic tests

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

Laboratory data

Normal<3 times ULN 3-5 times ULN >5 times ULN

Dynamic Tests

24hUFC -no./total no. (%)

2/93 (2.2) 19/93 (20.4) 7/93 (7.5) 65/93 (69.9)

8 a.m. ACTH– no./total no. (%)

5/102 (4.9) 48/102 (47.6) 24/102 (23.5) 25/102 (24.5)

no./total no.(%)

63/63 (100) 13/13 (100) 60/70 (85.7) 55/58 (94.8) 20/26 (76.9) 25/28 (89.3)

8 a.m. S-cortisol- no./total no. (%)

18/105 (17.1) 68/105 (64.8) 10/105 (9.5)

9/105 (8.6)

Overnight 1-mg DST (not suppressed) 2-mg DST (not suppressed)HDDST (not suppressed)CRH test (no response)

Desmopressin test (no response)IPSS (no gradient at baseline and after CRH)

ACTH, adrenocorticotropic hormone; ULN, upper limit of normal; UFC, urinary free cortisol; DST, dexamethasone; HDDST, high dose dexamethasone suppression test; CRH, corticotropin releasing hormone; IPPS, inferior petrosal sinus sampling.

eje@bioscientfica.com

For Review Only

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

Figure 1. Overall survival by neuroendocrine tumor (NET). BC: bronchial carcinoid; pNET, pancreatic NET, occult tumor. Number of patients at risk at 12, 24, 36, 48, 60 months are 36, 32, 27, 24, 21 (BC), 11, 10, 8, 6, 4 (pNET), 18, 16, 13, 10, 10 (Occult tumor). Survival probabilities were estimated employing the

Kaplan-Meier method. Survival curves were compared employing the log-rank test. Figure 1

175x175mm (300 x 300 DPI)

eje@bioscientfica.com

For Review Only

Figure 2. Overall survival by distant metastases. Number of patients at risk at 12, 24, 36, 48, 60 months are 21, 17, 10, 9, 7 (Yes), 57, 52, 45, 37, 31(No). Survival probabilities were estimated employing the Kaplan- Meier method. Survival curves were compared employing the log-rank test. Figure 2 175x175mm (300 x 300 DPI)

eje@bioscientfica.com

For Review Only

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository

Figure 3. Overall survival by 24-hr UFC levels. UFC: urinary free cortisol; ULN: upper limit of normal. Number of patients at risk at 12, 24, 36, 48, 60 months are 23, 20, 16, 14, 11 (up to 5 ULN), 40, 36, 32, 26, 21 (> 5 ULN). Survival probabilities were estimated employing the Kaplan-Meier method. Survival curves were compared employing the log-rank test. Figure 3 175x175mm (300 x 300 DPI)

eje@bioscientfica.com

For Review Only

Figure 4. Overall survival by neuroendocrine tumor (NET) surgery. Number of patients at risk at 12, 24, 36, 48, 60 months are 49, 46, 37, 33, 26 (Yes), 29, 23, 18, 13,12 (No). Survival probabilities were estimated employing the Kaplan-Meier method. Survival curves were compared employing the log-rank test. Figure 4 172x172mm (300 x 300 DPI)

eje@bioscientfica.com

iris-AperTO

University of Turin's Institutional Research Information System and Open Access Institutional Repository