clinicopathologic data were obtained through retrospective chart review. We identified 49 *PTEN* mutation-positive nodules from 48 patients. Patients were 57 years old on average (range 14-88) and 80% female. Cytology was predominantly indeterminate (73% atypia of undermined significance, 18% follicular neoplasm). There were 18 (29%) frameshift, 6 (10%) splice site, and 39 (62%) single nucleotide variant *PTEN* mutations. Fourteen (29%) nodules had two *PTEN* mutations, 5 (10%) had copy number alterations, and single cases had concurrent *BRAF K601N*, *EZH1*, and *NRAS* mutations. Surveillance was pursued for 27 (56%) and surgery for 21 (44%) patients (16 lobectomies, 5 total thyroidectomies). There were 14 follicular adenomas (FA), 4 oncocytic FA's, 1 oncocytic hyperplastic nodule, and 1 encapsulated follicular variant papillary thyroid carcinoma (EFVPTC). The EFVPTC had two low-frequency *PTEN* mutations, *PTEN* locus loss, an *NRAS* mutation, and was a low-risk tumor with capsular but no angiolymphatic invasion. Four (8.3%) patients had confirmed or suspected PHTS, all with multiple nodules. Two had surgery finding no malignancies (2 FA). One PHTS patient had a prior thyroidectomy for a *MET* mutation-positive nodule that was follicular carcinoma. On US, the mean nodule size of patients who had surgery was larger than the surveillance group (3.2 cm vs. 2.3 cm, p=0.02) but there was no difference in TI-RADS level (p=0.54). There was no difference in mean nodule size (3.5 cm vs. 2.6 cm, p=0.35) or TI-RADS level (p=0.81) between PHTS and non-PHTS patients. Among surveillance patients, follow-up US was done at 1 year in 13/19 (68%) and 2 years in 3/6 (50%) of eligible cases. Only 1/19 (5%) underwent repeat FNA for increased nodule size. No thyroid malignancy was found with a mean of 1.75 years of follow-up (range 1.00-2.78). The EFVPTC patient had no recurrence after 1.05 years of follow-up. In summary, thyroid nodules with isolated somatic *PTEN* mutations are primarily benign and can be safely followed with serial imaging. Nodules with multiple *PTEN* mutations were only associated with malignancy when accompanied by an additional *NRAS* mutation. About 8% of patients with *PTEN* mutations may be PHTS patients who may be at greater risk for malignancy.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Comparative Analysis of Different International Criteria (ACMG-AMP vs. TENGEN) Applied to Classification of Missense Germline Allelic Variants in Patients With Multiple Endocrine Neoplasia Type 1 or Suspected to this Syndrome.

Palloma C. Lugeiro, B.Sc., Betsaida Urtremari, MS, Lucas S. Santana, MS, Elisangela P S Quedas, MS, Delmar Muniz Lourenco, MD PhD.
University of Sao Paulo, School of Medicine, SAO PAULO, Brazil.

Context: Multiple endocrine neoplasia type 1 (MEN1) is a rare autosomal dominant genetic syndrome caused by germline pathogenic allele variants (PAV) in the *MEN1* tumor suppressor gene, which predispose *MEN1* carriers to the increased risk of several endocrine neoplasms throughout life. The *MEN1* gene (11q13), contains 10 exons encoding the MENIN protein. About 600 different PAVs have been reported, with 25% of them being missense variants. Of value, the definition of pathogenicity can be challenging, especially for missense variants. Thus, international guidelines for improving the classification of allele variants were recently defined by the ACMG-AMP (2015). Recently, applying ACMG-AMP criteria with inclusion of clinical features the TENGEN French group suggested modifications aiming to refine the classification of variants in MEN1 syndrome. **Objective:** To classify missense allelic variants found in the *MEN1* gene by the ACMG-AMP guideline using VARSOME and by the TENGEN group to support a comparative analysis of the results obtained with these two methodologies (ACMG-AMP; TENGEN). **Methods:** the classification of 16 different missense allele variants identified in 17 index cases with or suspected to MEN1 syndrome was conducted according to ACMG-AMP criteria using the VARSOME software followed by the analysis defined by the TENGEN group. **Results:** Of the 16 variants, 6 were new, 1 was recurrent (2 unrelated index cases) and 9 of them occurred in codons with previous reports of different amino acid exchanges in the same region. Differences observed in the classification by ACMG-AMP and TENGEN were: pathogenic variant (6% vs. 65%); probably pathogenic (88% vs. 12%) and variants of uncertain significance (VUS) (6% vs. 23%). The four VUS classified by TENGEN (one of them for ACMG-AMP) were of sporadic cases without clinical diagnosis of MEN1 (2, for one MEN1-related tumor in early age; 1, for suspected MEN1) or with high risk of phenocopy (1, HPT + acromegaly). **Conclusion:** The difference observed in the classification of the pathogenicity of these variants, especially due to the higher occurrence of VUS in TENGEN, indicates that the criteria adopted by ACMG-VARSOME would have to be refined for clinical features. By other side, TENGEN apparently reinforce the classification of pathogenicity in cases with clinical diagnosis of MEN1 and reduce the definition of pathogenicity to variants found in MEN1-suspected cases without clinical criteria for the MEN1 diagnosis. These protocols apparently need to be investigate, validated and, probably, improved in other cohorts to reduce risks of misinterpretations and classifications that can, lately, interfere in genetic counseling and in the clinical management of patients. Finally, long-term outcome of cases classified as VUS, functional studies and, familial segregation may reinforce the initial impressions obtained with TENGEN classification.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Comprehensive Analysis of Clinical Features in Index Cases With Multiple Endocrine Neoplasia Type 1 Refine the Risk Rate for Detection of Mutation Distinguishing Negative-Mutation (Phenocopies) and Positive-Mutation Cases.

Betsaida Urtremari, MS, Rafael A. Carvalho, MS, Lucas S. Santana, MS, Louise V. Antonio, B.Sc., Flavio G. Ribeiro, B.Sc., Palloma C. Lugeiro, B.Sc., Elisangela P S Quedas, MS, Delmar M. Lourenco Jr, MD PhD.
University of Sao Paulo, School of Medicine, Sao Paulo, Brazil.

Background: Index cases clinically diagnosed with multiple endocrine neoplasia type 1 (MEN1) are declared as MEN1 phenocopies if no germline *MEN1* mutation is identified. In comparison with positive-mutation cases, most phenocopies have been diagnosed in older age, mainly by association of primary hyperparathyroidism (HPT) and pituitary adenoma (PIT), with HPT predominantly diagnosed as uniglandular disease. Besides that, phenocopies rarely develop a third MEN1-related tumor and are associated with lower morbidity and longer survival. However, all these data are mainly derived of genetic studies by Sanger targeted to *MEN1* gene from a strict number of MEN1 phenocopies. **Objectives:** to recognize strong clinical profiles capable of predicting the occurrence or absence of germline *MEN1* mutation refining the clinical differentiation of phenocopies and mutation-positive cases before genetic testing disclosure. **Casuistic/Methods:** 143 MEN1 index cases: 87 *MEN1*-positive and 56 true MEN1 phenocopies (excluded mutations for MLPA assay and by a mini-painel based on long-range PCR and next generation sequencing of 6 MEN1-related genes (*MEN1, p15*, *p18*, *p21*, *p27*, *AIP*) covering full coding and non-coding regions. **Results:** High detectability rate of *MEN1* mutation was associated with the presence of ≥ 4 organs affected for primary tumors (100%), association of HPT/PET (neuroendocrine pancreatic tumor)/PIT (93%), HPT/PET (81%), positive familial history (88% vs. 48%), presence of PET (84%) as malign (80%) as multifocal (95%), two different PETs (100%), multiglandular HPT (81%) and diagnosis of one MEN1-related tumor (93%) or of two/three MEN1 tumors diagnosed before 21y (100%). The combination HPT/PIT has the lowest rate of detection of mutation (33%), it is even lower if PIT was acromegaly (12%) or age at the diagnosis of HPT and PIT was, respectively, > 45y and >30y (8%) and absent if it is added uniglandular HPT (0%) or if there was association HPT/PIT (age-independent) with uniglandular HPT (0%). The prediction for detection of mutation increases if these HPT (> 45y)/PIT (> 30y) cases have multiglandular HPT (20%) and it is 100% with association HPT (< 30y)/PIT (< 21y). A $p < 0.05$ was observed to all data above. **Conclusions:** By integration of phenotypic clues and full genetic analysis applied to the largest MEN1 phenocopy series, we identified strong clinical predictors capable of anticipate the potential risk rate for mutation detection revealing the estimated chance of an index case harbor a mutation or be classified as phenocopy. By their peculiarities, the management/treatment of phenocopies should potentially be different of that recommended to mutation-positive cases.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Development of a PTHrP Chemiluminescent Immunoassay to Assess Humoral Hypercalcemia of Malignancy

Susan Louise Ashrafzadeh-Kian, MS, Joshua Bornhorst, Ph.D., Alicia Algeciras-Schimnich, Ph.D.
Mayo Clinic, Rochester, MN, USA.

Background: Measurement of parathyroid hormone related peptide (PTHrP) is helpful in the diagnosis and clinical management of patients suspected of humoral hypercalcemia of malignancy (HHM). In these patients uncontrolled release of PTHrP by tumor cells is responsible for the hypercalcemia and PTH concentrations are typically suppressed.
Objective: Develop a sensitive and specific assay for quantitation of PTHrP in plasma.
Method: Calibrators (PTHrP 1-86) and samples (50uL) were incubated with an anti-PTHrP goat polyclonal acridinium ester labeled antibody. Complexes were transferred and incubated in a microplate coated with an anti-PTHrP polyclonal rabbit antibody. After washing, the acridinium ester generated signal, which is directly proportional to the amount of PTHrP in sample, was quantified.
Results: In this assay PTHrp was stable for 24 hours ambient, 3 days refrigerated, 34 days frozen and through 3 freeze/thaws. Intra and inter-assay imprecision in EDTA plasma (~0.16-35.0 pmol/L) ranged from 2.2-8.6% and 5-15%, respectively. The limit of detection was 0.04 pmol/L and the limit of quantitation was 0.16 pmol/L (15% CV). The analytical measuring range was 0.39-50.5 pmol/L (slope of 1.07 and r^2 of 0.99). Average spike recovery was 98% (range 85-108%). The assay was not affected by hemoglobin of ≤500 mg/dL, triglycerides of ≤2000 mg/dL, or bilirubin of ≤50mg/dL. No hook effect was noted up to 500 pmol/L. PTH (1-84) did not cross-react in the assay. C-terminal PTHrP(107-139), and N-terminal PTHrP(1-36) had no significant cross-reactivity (≤1.1%). Mid-PTHrP(38-94) had 8.3% cross-reactivity. Comparison with an in-house PTHrP assay (n=267) showed an r^2 of 0.96, and slope of 2.25 by Passing-Bablok regression fit. The 97.5% reference interval for PTHrP (n=114) was ≤0.7 pmol/L, however a higher concentration (≤4.2 pmol/L) was identified as a more specific clinical cut-off. A retrospective clinical validation study showed that using ≤4.2 pmol/L resulted in a 91% clinical sensitivity and a 98% clinical specificity.
Conclusion: We have developed an analytically and clinically sensitive and specific PTHrP immunoassay. A cutoff of ≤4.2 pmol/L is clinically useful in the evaluation of patients suspected of hypercalcemia of malignancy.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Effect of Conventional Chemotherapy in Patients With Neuroendocrine Tumours: A Systematic Review

Anida Divanovic, MD, MSc[1], Maralyn Rose Druce, MA MBBS FRCP PhD MMEd PFHEA[2].
[1]Clinical Center University of Sarajevo, Sarajevo, Bosnia and Herzegovina, [2]Barts and the London School of Medicine, QMUL, Herts, United Kingdom.

Background: Neuroendocrine tumours (NETs) are a wide-ranging group of neoplasms originating from neuroendocrine cells. In 2014 NETs incidence was 7 per 100000 people annually. The purpose of this systematic review is to evaluate the safety and efficacy of various types of chemotherapeutic agents on gastroenteropancreatic NETs (GEP NETs) and to determine which type of chemotherapy