Gynecologic Oncology 140 (2016) 486–493

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

First application of the Automated QUantitative Analysis (AQUA) technique to quantify PTEN protein expression in ovarian cancer: A correlative study of NCIC CTG OV.16☆

J.I. Weberpals [a,b,*], M.S. Amin [c], B.E. Chen [d], D. Tu [d], J.N. Spaans [a], J.A. Squire [e], E.A. Eisenhauer [d,f], S. Virk [d,g], D. Ma [h], M. Duciaume [a], P. Hoskins [i], D.P. LeBrun [g]

[a] Centre for Cancer Therapeutics, Ottawa Hospital Research Institute, Canada
[b] Division of Gynecologic Oncology, The Ottawa Hospital, Ottawa, Ontario, Canada
[c] Department of Laboratory Medicine, Mayo Clinic, Rochester, MN, USA
[d] NCIC-CTG, Kingston, Ontario, Canada
[e] Department of Pathology and Forensic Medicine, Ribeirão Preto Medical School, University of Sao Paulo, Ribeirão Preto, Brazil
[f] Department of Oncology, Queen's University, Kingston, Ontario, Canada,
[g] Department of Pathology and Molecular Medicine, Queen's University, Kingston, Ontario, Canada
[h] Department of Mathematics and Statistics, Queen's University, Kingston, Ontario, Canada
[i] BC Cancer Centre, Vancouver, British Columbia, Canada

HIGHLIGHTS

- AQUA is a viable option to measure protein expression in OC.
- PTEN quantification by AQUA associates with clinical and treatment outcomes in OC.
- Integration of PTEN expression as a biomarker requires study in select OC patients.

ARTICLE INFO

Article history:
Received 4 December 2015
Received in revised form 9 January 2016
Accepted 11 January 2016
Available online 13 January 2016

Keywords:
BRCA1
PTEN
Ovarian carcinoma
AQUA

ABSTRACT

Background. Platinum resistance is a dominant cause of poor outcomes in advanced ovarian cancer (OC). A mechanism of platinum resistance is the inhibition of apoptosis through phosphatidylinositol 3 kinase (PI3K) pathway activation. The role of phosphatase and tensin homolog (PTEN), a negative regulator of this pathway, as a tumor biomarker is unclear. Quantitative analysis of PTEN expression as an alternative to immunohistochemistry has not been considered.

Patients and methods. In 238 patient tumors from the NCIC-CTG trial OV.16, PTEN protein expression was quantified by Automated QUantitative Analysis (AQUA). Cox model was used to study the association between PTEN expression and clinical outcomes using a minimum *p*-value approach in univariate analysis. Multivariate analysis was used to adjust for clinical and pathological parameters.

Results. PTEN scores (range 13.9–192.3) of the 202 samples that passed quality control were analyzed. In univariate analysis, there was a trend suggesting an association between PTEN expression by AQUA as a binary variable (low ≤61 vs high >61) and progression free survival (HR = 0.77, $p = 0.083$), and in multivariate analysis, this association approached significance (HR = 0.74, $p = 0.059$). The relationship between quantitative PTEN expression and PFS differed ($p = 0.01$ for interaction) by the extent of surgical debulking (residual disease (RD) <1 cm or ≥1 cm), with a numerically superior PFS in patients with high PTEN (23.5 vs 14.9 m) only when RD < 1 cm ($p = 0.19$). There was no association between PTEN levels and overall survival.

Conclusions. AQUA is a novel method to measure PTEN expression. Further study of PTEN as a biomarker in OC is warranted.

Abbreviations: OC, epithelial ovarian cancer; PTEN, phosphatase and tensin homolog; AQUA, Automated QUantitative Analysis; RD, residual disease; OS, overall survival; PFS, progression free survival; RR, response rate; HR, hazard ratio.

☆ Funding: This work was supported by the Ontario Institute of Cancer Research (J.A.S.). The NCIC Clinical Trials Group receives programmatic funding by a grant from the Canadian Cancer Society.

* Corresponding author at: The Ottawa Hospital, General Campus, Division of Gynaecologic Oncology, Room 8130, 501 Smyth Road, Ottawa, Ontario K1H 8L6, Canada.
E-mail address: jweberpals@ottawahospital.on.ca (J.I. Weberpals).

http://dx.doi.org/10.1016/j.ygyno.2016.01.015
0090-8258/

1. Introduction

The survival rate in advanced stage ovarian carcinoma (OC) has seen only a modest improvement over the last several decades with fewer than 30% of patients surviving 5 years. [1] Debulking surgery and carboplatin/paclitaxel chemotherapy remain the standard first-line treatment. Despite a response rate of 75% [2], OC patients usually relapse, develop platinum resistance and eventually succumb to their disease. [3] While a number of factors, including the extent of surgical debulking, are known to affect the risk of early recurrence [4], the discovery of novel strategies to overcome platinum resistance with targeted therapies and predictive biomarkers of platinum drug response remain urgent priorities in OC.

As the end product of an accumulation of genetic and epigenetic alterations responsible for malignant growth, protein expression analysis is an attractive source for predictive biomarkers of treatment response. Although immunohistochemistry (IHC) is a popular and widely available technique to ascertain protein expression in formaldehyde-fixed paraffin-embedded (FFPE) tissue samples, as a subjective and semi-quantitative approach it suffers from relatively poor reproducibility. [5,6] The Automated QUantitative Analysis (AQUA) technique permits in situ assessment of histology sections prepared from tissue microarrays (TMAs) for unbiased quantification of proteins. [7] Using this technique, subcellular compartments are defined using molecular methods and the measurement of protein expression within these compartments translates to a number directly proportional to the number of molecules per unit area (the concentration) [8] and results in an objective and reproducible measure of protein expression.

In OC, a major cause of platinum resistance is the inhibition of platinum-induced apoptosis through activation of the phosphatidyl inositol 3 kinase (PI3K) pathway. [9] Tumors in at least one third of patients with high grade OC have activation of the PI3K pathway due to rare

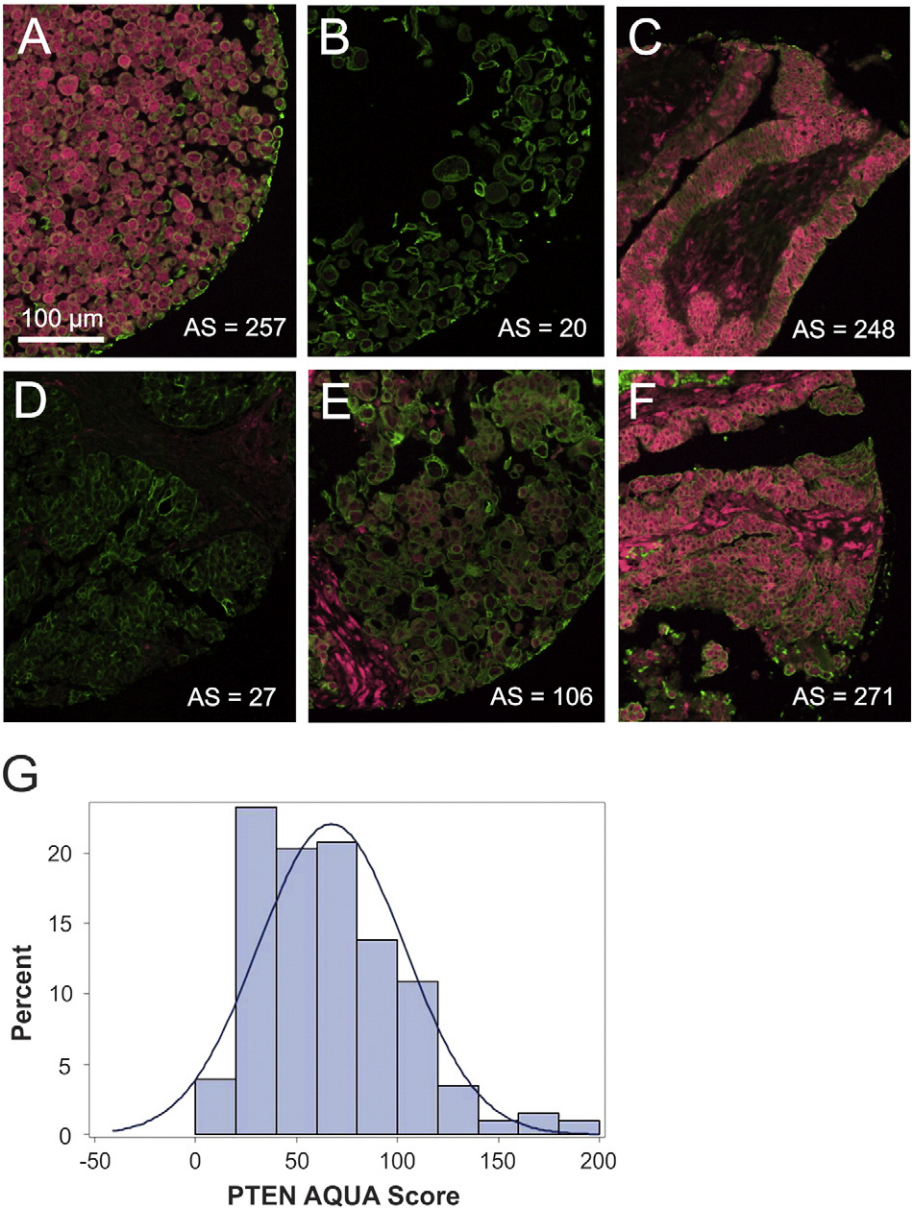

Fig. 1. Quantification of PTEN protein by immunofluorescence. (A) MCF7 cell line. (B) HCC1937 cell line. (C) Normal fallopian tube. (D, E, F) Ovarian carcinomas. Red, Cy5/PTEN; green, Alexa Fluor 488/keratin; AS, AQUA score. Note that the subcellular localization of PTEN ranges from primary nuclear (A and E) to primary cytoplasmic (F) and that PTEN is expressed frequently in stromal fibroblasts (C, D, E and F). (G) Histogram showing the normal distribution of the AQUA scores from 202 ovarian carcinoma samples.