INTERPRETATION OF IMMUNOHISTOCHEMICAL STAINS - DIFFICULTIES AND PITFALLS

Gabor Fischer
Diagnostic Services Manitoba
University of Manitoba

The cQc and Diagnostic Services Manitoba
Joint Symposium on Shaping the Future of Quality Improvements in Pathology

June 10-12, 2016
Radisson Hotel Downtown
288 Portage Avenue
Winnipeg, Manitoba
Diagnostic Services Manitoba

Number of pathologists: 48
  surgical pathologists, neuropathologists, autopsy pathologists, hematopathologists

AP residency training program: 11 residents

Number of surgical cases: ~99,000

Number non-gynecologic cytology cases: ~19,000
IHC INTERPRETATIONS – LOCAL DATA

How many immunostains do we order per year?

57,184 (2015)

What is the most commonly ordered IHC?

pancytokeratin (CK AE1/AE3): 3358 (5.9%)

Followed by

ki67 (2166)
CD3 (1877)
p63 (1807)
CD20 (1672)
INCREASED UTILIZATION OF IMMUNOSTAINS

Increased utilization, verification, and clinical implications of immunocytochemistry: Experience in a northern New England hospital

Jennifer L. Sauter M.D., Abiy B. Ambaye M.D., Sharon L. Mount M.D.

Diagnostic Cytopathology
2015;43:688–695

Usefulness of Immunohistochemical and Histochemical Studies in the Classification of Lung Adenocarcinoma and Squamous Cell Carcinoma in Cytologic Specimens

Rebecca Oece, MD, Naobumi Tochigi, MD, N. Paul Ohori, MD, and Sanja Dacic, MD, PhD

American Journal of Clinical Pathology
2011;136:81-87

Number of cytology cases in which immunostains were ordered increased more than 3x in a 4-year interval (2007-11)

The introduction of targeted therapies led to a 600% increase in IHC utilization to diagnose lung squamous cell and adenocarcinomas (combined cytology and biopsy specimens, before and after 2005)
Technical requirements, protocols, preparations have to be based strictly on “science”

Interpretation has its `rules, but it has an intuitive component (“art”)
Pathologists, residents, students responded

The “correct” answer is 7.5%

Wide range of responses

43 pathologists: 0-100%
mean: 29.7%

“Please read the following hypothetical scenario: An experienced histopathologist after examining a haematoxylin and eosin stained slide and taking into account the clinical and radiological context of the patient considers there to be a 99% chance that the patient has tumour A and a 1% chance that they have tumour B. These tumours require different treatment. Antibody X has been shown to be positive in 80% of cases of tumour B, and negative in 90% of cases of tumour A. The pathologist is surprised to find that the lesion stains convincingly for antibody X. What would you estimate are the chances that the patient has tumour B? (Please state estimated percentage)”
INTUITION VS. SCIENCE IN THE IHC WORLD

Study has many limitations
  we don`t rank exact probabilities (%) based on morphology

The antibodies can determine the probability of a given diagnosis
  Antibody A is positive in X % of the cases, B is in Y% of the cases

We don`t calculate these probabilities in a routine practice

We synthesize all available information
  Intuitive estimation may be very far from mathematical calculation
DISCREPANCIES IN REPORTING IHC RESULTS

May be related to:
- technical issues
  - fixation
  - processing
  - reagents
  - instruments
  - methodology
- or interpretation

Technical and methodological aspects are well investigated.

Interpretation/evaluation receives less attention.
Two potential problems

Interpretation of the immunostain
  
  Negative stain is interpreted as positive or a positive as negative
  
  May or may not affect the final diagnostic interpretation

Final diagnostic interpretation
  
  The stain is interpreted correctly, but it leads to an incorrect conclusion
Aberrant E-cadherin expression in lobular breast carcinomas

CAUSES OF DISCREPANT IHC INTERPRETATIONS

Lack of definition of “positive”

- Applying the cut-off numbers and scoring systems when appropriate

Staining intensity

- Weak, ambiguous – are they strong enough to call them positive?

Area to assess the stain

- Random vs. central vs. peripheral
- Solid vs. necrotic
- Invasive vs. in situ tumor cells
- Tumor cell vs. not

Staining patterns (cytoplasmic, nuclear, membranous, dot-like)

Misleading literature

Inter- and intraobserver variability
WHAT IS POSITIVITY?

Definition and threshold issue

Intensity – qualitative component

What staining intensity is considered to be positive?

Extent – quantitative component

What percentage of the cells have to be positive?

only 1 cell? or a certain% of the cells?

Do you have to combine the two and if so, based on what guidelines?
WHAT IS POSITIVITY?

Different for diagnostic and prognostic interpretations

Diagnostic – Class I
- Sometimes one positive cell can be enough
  - CMV, Herpes
- In many scenarios the threshold is not well-defined
  - high interobserver variability
  - vague, ambiguous, non-contributory
- Clear, strong positivity is not an issue

Prognostic biomarkers – Class II
- Should follow well defined guidelines
AREAS TO BE ASSESSED

Highest proliferative invasive area at the periphery of the tumor
  for mitotic index, proliferation markers

Invasive vs. in-situ
  Her2 – invasive

Necrosis
  nonspecific background staining

Poorly differentiated areas within the tumor
  for prognostic markers

Well-differentiated areas
  for diagnostic applications (typical patterns are better preserved)

Napsin A was introduced as a marker for lung adenocarcinoma.

Some papers reported positivity in squamous cell carcinomas (up to 26%).

Really??? It would be a major problem…
90 lung squamous cell carcinomas

All negative for Napsin A

However alveolar macrophages and type 2 pneumocytes are often positive

Can be really difficult in biopsies or TMA studies
WHAT CELLS ARE POSITIVE?

Gastrointestinal spindle cell tumor

H&E  CD117  DOG-1  Tryptase  SMA

Ye JX et al. Histol Histopathol. 2015 May;30(5):581-8
Mast cells can be “passenger” cells in spindle cell lesions

Mast cells are positive for CD 117

Mast cells can be spindled or ovoid

DOG-1 stains ICC (Interstitial Cells of Cajal)

ICCs may be hyperplastic in leiomyomas
LOCALIZATION OF IMMUNOSTAINS AT THE CELLULAR LEVEL

Membranous

Cell adhesion molecules: E-cadherin, CD56, Ber-EP4

Cell surface/transmembrane receptors/proteins: CD10, CEA, most leukocyte antigens (CD3 and CD20), EMA, CD117

Proteins linking surface molecules to cytoskeleton: β-catenin, dystrophin

Cytoplasmic – different patterns

Granular: chromogranin, HMB45

Fibrillar: intermediate filaments (desmin, cytokeratin, vimentin)
  may appear membranous, due to condensation beneath membrane

Diffuse: myoglobin, thyroglobulin
LOCALIZATION OF IMMUNOSTAINS AT THE CELLULAR LEVEL

Nuclear

Cell-cycle associated proteins: \textit{ki67, p16}
Transcription factors: \textit{TTF-1, CDX-2, myogenin, PAX-5}
Tumor suppressor genes: \textit{p53, p63, WT-1, Rb}
Nuclear enzymes and proteins: \textit{TdT, mismatch products}
Steroid hormone receptors: \textit{ER/PR}
Calcium-binding proteins: \textit{S-100, calretinin}
also show cytoplasmic pattern
Some viral proteins: \textit{CMV, herpes}
INCONSISTENT STANDARDS FOR PUBLICATIONS

Misleading literature: potential source of incorrect diagnostic interpretation

Incorrect information may lead to a misdiagnosis and a lawsuit

Publications with IHC results should have high basic standards*

- distribution of the staining: membranous, cytoplasmic, nuclear
- consistency and quality of the positivity: diffuse, granular, fibrillary
- proportion of positively stained cells
- relevant variations in staining intensity
- cutoff levels for positive interpretations
- information about positive and negative controls
- information about the observers (pathologists vs. not)

THE CHANGING WORLD OF IHC MARKERS

New ICH stains are marketed to address a specific need

The final role of the markers in practice may be different

  accumulation of evidence takes years (sensitivity and specificity)

  some stains perform below expectations, others do well

  some stains deliver unexpected advantages

Healthy scepticism helps when we read about a new marker

Continuous learning is essential
THE CHANGING WORLD OF IHC MARKERS

Journey of an antibody
   Expanding or diminishing roles

New generation antibodies for organ specific scenarios
   Advantages and pitfalls

Changing recommended panels for differential diagnostic scenarios
THE CHANGING ROLE OF IHC MARKERS

RCC – not really specific for renal cell carcinoma

TTF1 – less sensitive and specific for lung adenocarcinoma than we thought

CK5/6 – became very useful for intraductal proliferations in breast

CD117 – useful marker for seminoma, mast cells

CA19-9 – not specific for pancreatic origin

CA125 – not a specific Mullerian marker

CK19 - not specific for pancreatobiliary origin
CA 125

NOT specific for Mullerian origin

<table>
<thead>
<tr>
<th>Tumor site</th>
<th>CA 125 positivity rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovary</td>
<td>98%</td>
</tr>
<tr>
<td>Endometrium</td>
<td>93%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>82%</td>
</tr>
<tr>
<td>Lung</td>
<td>66%</td>
</tr>
<tr>
<td>Cervix</td>
<td>64%</td>
</tr>
<tr>
<td>Thyroid</td>
<td>50%</td>
</tr>
<tr>
<td>Breast</td>
<td>35%</td>
</tr>
</tbody>
</table>

Gremel G. et al, Histopathology; 64: 293-305
NOT specific for pancreatic origin

<table>
<thead>
<tr>
<th>Tumor site</th>
<th>CK19 positivity rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholangiocarcinoma</td>
<td>100%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>100%</td>
</tr>
<tr>
<td>Gallbladder</td>
<td>100%</td>
</tr>
<tr>
<td>Lung</td>
<td>100%</td>
</tr>
<tr>
<td>Colon</td>
<td>100%</td>
</tr>
<tr>
<td>Ovary</td>
<td>100%</td>
</tr>
<tr>
<td>Cervix</td>
<td>95%</td>
</tr>
<tr>
<td>Urothelial</td>
<td>95%</td>
</tr>
</tbody>
</table>
40-year-old woman with a rubbery uterine mass (4.5 cm)
HMB-45

Signed out as uterine angiomyolipoma
Call from the clinician – Really? Are you sure? Can you send it out?

Pathol Int. 2001 Nov;51(11):896-901.

Uterine angiomyolipoma: case report and review of the literature.

Yaeqashi H¹, Moriya T, Soeda S, Yonemoto Y, Nagura H, Sasano H

Author information

Abstract
Extrarenal angiomyolipomas (AML) have been reported at various anatomical sites, but infrequently in the gynecological region. In the uterus, only a few cases have been described. We describe a uterine angiomyolipoma occurring in a 40-year-old woman without evidence of tuberous sclerosis. The tumor arose on the right wall of the uterine body and was partially cystic, and it was associated with marked degeneration. It was composed of mature adipose tissue, anomalous blood vessels and non-vascular smooth muscle cells. Immunohistochemistry revealed that non-vascular smooth muscle cells were positive for alpha-smooth muscle actin (alpha-SMA), desmin, vimentin, antihuman muscle actin (HHF35) and progesterone receptor (PR), and negative for cytokeratin, antihuman melanoma (HMB45), CD34, S-100 and estrogen receptor (ER). It is of particular interest that non-vascular smooth muscle cells were negative for HMB45, in contrast to renal and other extrarenal AML in which HMB45 immunoreactivity has been demonstrated in these cells.
THE JOURNEY OF HMB 45

A distinctive translocation carcinoma of the kidney; “rosette forming,” t(6;11), HMB45-positive renal tumor: a histomorphologic, immunohistochemical, ultrastructural, and molecular genetic study of 4 cases☆,☆☆

Fredrik Petersson MD, PhD a,b, Tomáš Vaněček PhD a, Michal Michal MD a, Guido Martignoni MD, PhD c, Matteo Brunelli MD, PhD c, Zbyněk Halbhuber MD d, Dominic Spagnolo MD, PhD e, Naoto Kuroda MD, PhD f, Ximing Yang MD, PhD g, Isabel Alvarado Cabrero MD h, Milan Hora MD, PhD i, Jindřich Branžovský a, Sandra Trivunic MD j, Denisa Kacerovská MD, PhD a, Petr Steiner MSc a, Ondřej Hes MD, PhD a,*
The Lung-Restricted Marker Napsin A Is Highly Expressed in Clear Cell Carcinomas of the Ovary

Patricia L. Kandalaft, MD,¹ Allen M. Gown, MD,¹ and Christina Isacson, MD²

From ¹PhenoPath Laboratories, PLLC, Seattle, WA, and ²CellNetix Pathology and Laboratories, Seattle, WA.

Key Words: Napsin A; Ovarian clear cell carcinoma; Immunohistochemistry

Am J Clin Pathol December 2014;142:830-836

DOI: 10.1309/AJCP8W2E01AHSOF
Applications and Limitations of Immunohistochemical Expression of “Napsin-A” in Distinguishing Lung Adenocarcinoma From Adenocarcinomas of Other Organs

Maryam Kadiyar, MD* and Behnaz Boozari, MD†
Criteria for staining evaluation:

Only coarse granular cytoplasmic staining was considered positive

Scoring: based on intensity and percentages of positive cells

All scores >2 were considered positive
<table>
<thead>
<tr>
<th>Tumor site</th>
<th>Napsin-A positivity rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung adenocarcinoma</td>
<td>100%</td>
</tr>
<tr>
<td>Renal cell carcinoma, papillary</td>
<td>87%</td>
</tr>
<tr>
<td>Thyroid papillary carcinoma</td>
<td>48%</td>
</tr>
<tr>
<td>Renal cell carcinoma, clear</td>
<td>29%</td>
</tr>
<tr>
<td>Endometrioid adenocarcinoma</td>
<td>10%</td>
</tr>
<tr>
<td>Lung squamous cell carcinoma</td>
<td>0%</td>
</tr>
<tr>
<td>Breast ductal carcinoma</td>
<td>0%</td>
</tr>
<tr>
<td>Colon adenocarcinoma</td>
<td>0%</td>
</tr>
</tbody>
</table>
TTF-1 SPECIFICITY

62-year-old man, liver mass lesions, unknown primary
TTF-1 SPECIFICITY

Aberrant expression rate is clone dependent
8G7G3/I: around 2%, SPT24: 10% or higher (colon)
BIRTH OF AN ANTIBODY

SOX-10

GATA-3

PAX-8

P40

Search ID: amrn639
DEATH OF AN ANTIBODY

NEURON SPECIFIC ENOLASE

R.I.P.

NSE
BREAST MARKERS

ER
Mammoglobin
GCDFP-15

50% of metastatic breast carcinomas are negative for all 3

GATA-3

positive in 96% of metastatic breast carcinomas

GATA 3 – A MULTISPECIFIC BUT POTENTIALLY USEFUL MARKER IN SURGICAL PATHOLOGY – A SYSTEMATIC ANALYSIS OF 2500 EPITHELIAL AND NON-EPITHELIAL TUMORS

Markku Miettinen, MD1, Peter A. Mc. Cue, MD2, Maarit Sarlomo-Rikala, MD3, Janusz Rys, MD4, Piotr Czapiewski, MD5, Krzysztof Wazny, MD5, Renata Langfort, MD5, Piotr Wałoszczyk, MD7, Wojciech Biernat, MD5, Jerzy Lasota, MD1, and Zengieng Wang, PhD1

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>choriocarcinoma</td>
<td>100%</td>
</tr>
<tr>
<td>urothelial carcinoma</td>
<td>84% - 100%</td>
</tr>
<tr>
<td>chromophobe RCC</td>
<td>51%</td>
</tr>
<tr>
<td>cholangiocarcinoma</td>
<td>9%</td>
</tr>
<tr>
<td>lung adenocarcinoma</td>
<td>9%</td>
</tr>
</tbody>
</table>
PITFALLS IN PROSTATE IHC INTERPRETATION

Scenarios related to certain IHC stains

Basal cell markers

Prostate specific markers
PITFALLS IN PROSTATE IHC INTERPRETATION

Racemase (AMACR): intense cytoplasmic granular positivity in carcinoma
frequently overexpressed in HGPIN

certain carcinoma types may not overexpress AMACR
atrophic, hormon treated, foamy gland

benign mimickers may overexpress it
adenosis, atrophy, hyperplasia

never interpret AMACR in isolation

don`t use it as a prostate marker
present in colon, lung, breast, kidney, ovary, bladder
PITFALLS IN PROSTATE IHC INTERPRETATION

HGPIN

Atrophy
PITFALLS IN PROSTATE IHC INTERPRETATION

Basal cell markers can be negative in benign mimickers

- adenosis
  - staining may be minimal, racemase may not help
  - “atypical glandular proliferation, adenosis can not be excluded”

- partial atrophy
  - go with morphology
  - staining can completely mimic carcinoma (-basal, +AMACR)

False positive staining of adenocarcinoma for basal cell markers

- extremely rare, but can happen in Gleason 3 patterns
- usually patchy, may be diffuse (for both 34BE12 and p63)
PITFALLS IN PROSTATE IHC INTERPRETATION

Gleason 3 with HMWCK positivity
High grade prostatic adenocarcinoma vs. invasive urothelial carcinoma

Recommended panel: 2 prostatic and 2 urothelial markers

<table>
<thead>
<tr>
<th>Prostate marker</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA</td>
<td>specific / cytoplasmic granular</td>
</tr>
<tr>
<td>PSAP</td>
<td>specific / cytoplasmic granular</td>
</tr>
<tr>
<td>PSMA</td>
<td>sensitive, but stains 17% of urothelial carcinomas</td>
</tr>
<tr>
<td></td>
<td>prostate: cytoplasmic + apical or membranous non-prostate: cytoplasmic only</td>
</tr>
<tr>
<td>Prostein (p501S)</td>
<td>specific / perinuclear</td>
</tr>
</tbody>
</table>
PITFALLS IN PROSTATE IHC INTERPRETATION

High grade prostatic adenocarcinoma vs. invasive urothelial carcinoma

Recommended panel: 2 prostatic and 2 urothelial markers

<table>
<thead>
<tr>
<th>Urothelial marker</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>GATA-3</td>
<td>very specific, less sensitive</td>
</tr>
<tr>
<td>34BE12</td>
<td>sensitive, but may stain PD prostate carcinomas</td>
</tr>
<tr>
<td>p63</td>
<td>specific, less sensitive</td>
</tr>
<tr>
<td>Uroplakin, thrombomodulin</td>
<td>specific / less sensitive</td>
</tr>
</tbody>
</table>
73-year-old woman with a 4 cm thyroid mass and compressive symptoms
Lesions in brain, lung, adrenal and lymph nodes
Thyroid FNA + immunostains: thyroglobulin (+), TTF-1 (+) and calcitonin (-)

Diagnosed as poorly differentiated (insular) thyroid carcinoma

Total thyroidectomy + lymph node dissection to relieve symptoms + diagnosis
Thyroid shows tumor foci with glandular and papillary features
Thyroid and lymph nodes show different IHC patterns (thyroglobulin + vs -)
Diagnosis was changed to primary lung adenocarcinoma with widespread metastases (including thyroid)

Patient died 7 months later

Thyroglobulin was not expressed by the tumor cells

Nonspecific uptake and staining due to diffusion artefact from the surrounding follicles? from the needle as it punctured through the follicles?
TMA (tissue microarray) selected from radical prostatectomies

All stained for PDX-1 (pancreatic duodenal homeobox-1)

- Transcription factor overexpressed in prostatic adenocarcinoma
- Expressed in several types of carcinomas (gastric, pancreatic, prostate)
- Cytoplasmic stain
INTRA - AND INTEROBSERVER VARIABILITY

Stains evaluated by 4 independent observers
   2 pathologists with interest in GU pathology
   2 medical doctors with no formal training in pathology

Stains were scored twice by each participants
   2-week interval between the reads

Intra – and interobserver reproducibility was recorded

Time spent with slides was also recorded
INTRA - AND INTEROBSERVER VARIABILITY

Scoring: Intensity: 0-3  (0: no staining, 3: most intense)
Extent: 1-3  (1: ≤ 33%, 2: 34-66%, 3: ≥67%)
Two scores are multiplied (final score: 0-9)
### Table 3

Interobserver agreement (weighted kappa) between the four observers

<table>
<thead>
<tr>
<th>Observer</th>
<th>Intensity</th>
<th>Extent</th>
<th>IRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 vs. 2</td>
<td>0.807</td>
<td>0.245</td>
<td>0.794</td>
</tr>
<tr>
<td>1 vs. 3</td>
<td>0.799</td>
<td>0.255</td>
<td>0.882</td>
</tr>
<tr>
<td>1 vs. 4</td>
<td>0.842</td>
<td>0.206</td>
<td>0.802</td>
</tr>
<tr>
<td>2 vs. 3</td>
<td>0.771</td>
<td>0.216</td>
<td>0.770</td>
</tr>
<tr>
<td>2 vs. 4</td>
<td>0.817</td>
<td>0.109</td>
<td>0.671</td>
</tr>
<tr>
<td>3 vs. 4</td>
<td>0.786</td>
<td>0.250</td>
<td>0.817</td>
</tr>
<tr>
<td>Average</td>
<td>0.804</td>
<td>0.214</td>
<td>0.789</td>
</tr>
</tbody>
</table>

*IRP* immunoreactivity product (the product of intensity and extent)
INTRA - AND INTEROBSERVER VARIABILITY

Staining intensity – good results
  intraobserver: very high agreement
  interobserver: high (from substantial to very high)

Extent of staining – terrible numbers
  intraobserver: poor agreement
  interobserver: poor agreement

Non-pathologists spent more time on evaluation (2x)
  improved in the second run
Crowdsourcing IHC interpretation experiment

Outsourcing of tasks typically performed by experts to a large crowd

Kasparov vs. the World chess game (1999, internet)

- Plurality vote decided World Team’s moves - 50,000 people from 75 countries
- Kasparov won with whites
- He had “never expended as much effort on any other game in his life”

Crowdsourcing experiments in medicine

- Retinal fundus photography classification, malaria parasite quantification
- IHC experiment from Italy*
  - 13 breast images with MIB1 immunostain (positive-negative)
  - Crowd: 28 respondents from 18 countries, non-pathologists
  - Pathologists’ count is gold standard

In this task you are requested to click on all positive and negative cell nuclei, which are distinguishable for their color: positive nuclei are brown blobs, negative nuclei are blue blobs. To identify them, you have to click on the positive or negative buttons just below the image, and then click over the nuclei (after having selected one button, you may click on many nuclei of the same kind). If you change your mind about a nucleus, you can click again on it and delete your previous identification. Very small or very faint nuclei (compared to the rest) should not be counted. The image is longer than the screen, so you have to scroll down to see it completely. In the example image, positive nuclei are identified with an orange X, negative with a blue N, while the red X shows nuclei not to be counted because too faint or too small.
Crowdsourced median percentage is similar to gold standard

Counting is time-consuming by experts, may be difficult by software

Authors` conclusion: “method may be more aimed to research than routine”

When large number of images need ad hoc evaluation

<table>
<thead>
<tr>
<th>Image</th>
<th>Gold standard</th>
<th>Crowdsourced</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65.83%</td>
<td>69.62%</td>
</tr>
<tr>
<td>2</td>
<td>73.43%</td>
<td>69.10%</td>
</tr>
<tr>
<td>3</td>
<td>47.06%</td>
<td>41.26%</td>
</tr>
<tr>
<td>4</td>
<td>18.33%</td>
<td>23.14%</td>
</tr>
<tr>
<td>5</td>
<td>75.00%</td>
<td>68.83%</td>
</tr>
<tr>
<td>6</td>
<td>67.11%</td>
<td>61.90%</td>
</tr>
<tr>
<td>7</td>
<td>7.88%</td>
<td>12.63%</td>
</tr>
<tr>
<td>8</td>
<td>6.78%</td>
<td>8.25%</td>
</tr>
<tr>
<td>9</td>
<td>7.48%</td>
<td>10.71%</td>
</tr>
<tr>
<td>10</td>
<td>8.33%</td>
<td>10.05%</td>
</tr>
<tr>
<td>11</td>
<td>2.78%</td>
<td>2.88%</td>
</tr>
<tr>
<td>12</td>
<td>46.34%</td>
<td>42.66%</td>
</tr>
<tr>
<td>13</td>
<td>57.97%</td>
<td>45.23%</td>
</tr>
</tbody>
</table>
THANK YOU!

QUESTIONS?