EUROPEAN CONGRESS OF PATHOLOGY

Symposium - History of Pathology: Colours of Pathology.

Immunohistology: a historical perspective.

A Historical Perspective in 12 MILESTONES

1. “Invention of the pathologist”
2. Invention of Stains
3. Immunofluorescence
4. Peroxidase conjugates
5. Paraffin section – immunoperoxidase
6. PAP / ABC - high sensitivity detection
7. Monoclonal antibodies - hybridoma technique
8. Value in diagnosis - anaplastic tumors
9. Antigen Retrieval
10. Total Test – Automation - Reagents -- Quality
11. Biomarkers - Prognostic / predictive
12. Digitization / Image analysis / Quantification
It is to some degree a personal history—
For these “Milestones” have been along the road that I chanced along.

You will have travelled different roads;
but the important milestones are the same for all.

“Begin at he beginning
Go on until you get to the end
Then stop.”

The King to Alice
In wonderland
1850 HISTOLOGY
the microscope
“invented” the pathologist

1900 Immunity - antisera

1935 Fluorescent labels
    Frozen sections

1975 Immunoperoxidase
    Dx IHC on Paraffin sections
    Monoclonal antibodies

2000 Biomarkers
    Digital pathology

Following images from
Magic to Molecules:
A History of Disease and Pathology
Van den Tweel, Gu, Taylor, 2016
Leeuwenhoek - ‘animalicules’ 1632-1723

Medical use - 1840 s.
Joseph Jackson Lister
A wine merchant.
- corrected spherical aberration

150 YEARS In gestation

The Impact of New Technology

The Royal Microscopical Society 1839
John Hunter, 1728-1793

a surgeon. - behaved like a morbid anatomist / pathologist –

- did 10,000 + ‘autopsies’
- made 13,000+ specimens

-HAD A MICROSCOPE BUT WAS “Unimpressed by microscopists” -

Collection in 1842, Hunterian Museum
Now Royal College of Surgeons. London.
Hodgkin had a microscope - but did not use it in his 1832 PAPER.
1845 Case of Hypertrophy of the Spleen and Liver
in which Death Took Place from Suppuration of the Blood
Leucocytthemia - probably first published case of leukemia.

AND – in a decade everything changed
Sir James Paget. 1854

‘Lectures on Surgical Pathology’, based on a series of 36 lectures given at the College of Surgeons 1847-1852.

Rudolf Virchow 1858

Both

Classified cancers

Depicted cancer cells
“Thus the first pathologists emerged from the treacherous swamps of medieval practice on to the relatively firm ground that histopathology seemed to offer----”

Taylor CR Immunomicroscopy 1986

1860 onward
Ushered in the Age of the Microscope - for 150 years
THE Diagnosis was by H&E
- image analysis by mind and microscope !!! 1850 - 2019
From plants or the clothing dye industry

HISTOCHEMICAL STAINS
- Mallo
- Van Gieson
- Congo red
- Gram
- Ziehl-Neelsen
- Methylene blue
- Hematoxylin & eosin
- Hematoxylin & eosin
- Carmine

ANILINE DYES
- Congo red
- Acid fuchsin; Orange G
- Safranin
- Eosin; Methyl violet
- Basic fuchsin
- Aniline purple

1850 1860 1870 1880 1890 1900
For 150 + yrs - H&E - formalin paraffin section – diagnostic opinion by a histopathologist

1939.
Immunofluorescent labeling – on frozen sections
Coons, Fagraeus

1974
Immunoperoxidase - on FFPE tissue in ‘routine Dx’
Taylor, Burns, Mason et al Oxford
Albert Hewett Coons
Charite Krankenhaus in Berlin, 1939, during a 6 m break in his resident training at Mass General

Immunofluorescence labeling – on frozen sections

Countway Library, Harvard Medical School

In Berlin - Coons had ‘time to think’; while considering the Aschoff Nodule as a

“local hypersensitivity reaction involving components of the Group A hemolytic streptococcus.”

“It struck me that this theory had never been tested and indeed could not be tested without the demonstration of antibody or antigen, preferably both, in the local lesions.”

“The notion of labeling an antibody molecule with a visible label was perfectly obvious in this context.”


Karolinska.


Antibody Production in relation to the development of plasma cells.
Acta Medica Scandinavica

Beginnings of experimental IMMUNOPATHOLOGY

(Statens Bakteriologiska Laboratorium, SBL) 1944-47.
Spring picnic – Peter Biberfeld
by 1900 - H&E - formalin paraffin section - MORPHOLOGY

H&E became the GOLD STANDARD

1974 - these 2 problems solved
Immunoperoxidase on FFPE tissue
Combined immunology with morphology
(note also first ‘multiplex’ IHC stain)

1939
Immunofluorescent labeling

Huge experimental impact
BUT Limited use in DIAGNOSTIC histology –
1. Dark field microscopy
2. Frozen section
Meant loss of GOLD STANDARD MORPHOLOGY


U. Colorado School of Medicine, Medical Research Institute at Tokai University, Nagasaki University School of Medicine. Cal Poly.


Difference between night -------- and day!!!
1974.
Found its first application on routine formalin paraffin sections for IgG in lymphoma and Hodgkin’s disease.


Leading to 100s of papers in next 5 years. - not all supportive!!!
Jerry Garvin and friends

Association Of Pathology Chairs

Puerta Vallarta, Mexico. 1998

At first we could not detect much in FFPE
1. Poor detection methods
2. Poor antibodies (antisera)
3. Loss of antigenicity in FFPE

Ludwig Sternberger
Edgewood Arsenal, U Rochester, NY.

PAP
peroxidase-anti-peroxidase
More ‘sensitive’ Methods

AlkPAP & PAP

ABC


Now largely obsolete in Diagnostic work
Today - 40 yrs later no shortage of ‘sensitive’ high quality methods.

Hybridomas. -- a method of investigating --DNA to protein

Georges J. F. Köhler
1946-1995

César Milstein
1927-2002

Nobel Prize in Physiology and Medicine
1984

Cambridge
Following on from ------
Protein sequencing Sanger
DNA structure Crick and Watson
DNA sequencing Sanger

At Cambridge from
Max Planck Institute
For Immunobiology,
Freiburg.

British medical Council
Laboratory for Molecular
Biology, Cambridge
David York Mason

Returned to Oxford 1974
In next year doubled the World literature with half a dozen papers extending the range of antisera:
All IG  light and heavy chains, 
Lysozyme, muramidase
Lactoferrin
Blood Group A  Ag.

Critically he was one of first to see and exploit potential of hybridomas
In immunohistology

“At play we were owners of a small boat on the Oxford canal…christened the ‘Sanderson Polster’ in recognition of all the histology David would admit to knowing.”

As a result, today there is no shortage of antibodies:
In fact – the problem is almost that there are so many that QC and choice is difficult.
Anaplastic tumors

Small cell CARCINOMA vs Malignant LYMPHOMA

Bad prognosis vs Good prognosis
No effective Rx vs Effective Rx

Effects of wrong treatment
Cost of wrong treatment
Cost of further investigations
Deaths due to wrong treatment

Huge IMPACT For Diagnosis
At first IHC on FFPE struggled – most papers tried to prove it did not work

Kevin Gatter, Oxford student, then faculty was brave!

H&E diagnosis of anaplastic cancer WRONG in > 50% !!!

120 consecutive routinely processed biopsy specimens
unclassifiable (24 cases)
carcinomas (43 cases)
lymphomas (53 cases).

With IHC – “Lymphoma accounted for 29 of the 43 cases initially thought to be carcinomas.”

Gatter KC et al.
Clinical importance of analyzing malignant tumours of uncertain origin with immunohistochemical techniques

The clever men at Oxford, Knowed all that there was to be knowed, But none of them knew half as much As intelligent Mr Toad.
Wind in the Willows. K Grahame
But formalin was still a problem
- Loss of antigenicity  AND
- FFPE is NOT standardized
---- Time of fixation
---- Thickness of tissue block
---- pH and ‘freshness’ of formalin

“the reason I like formalin fixed tissue for IHC –
is because that’s what there is.”

Favorite heresies  # 63

Guillermo Herrera.  Birmingham, Alabama.
IHC Workshop 1995
Anaplastic tumour of unknown origin: carcinoma; lymphoma; melanoma; sarcoma?

"Negative" for keratin.

1991. Same case
Same method

PLUS
ANTIGEN RETRIEVAL
Antigen retrieval
epitope retrieval
unmasking
uncloaking
--replaced enzymatic digestion, which was less effective and difficult to control

Shan Rong Shi

Based upon work of Fraenkel-Conrat and colleagues, 1940s,
----- restoring effectiveness of formalin treated tetanus toxoid vaccine by boiling.

CONCERNS. - it is not just the antibody not just fixation

As early as 1979 - Workshops sponsored by - NIH, Biologic Stain Commission and the FDA

1992. Total Test in IHC


Table 1. Components of the Total Test in Immunohistochemistry

<table>
<thead>
<tr>
<th>Element of Testing Process</th>
<th>Quality Assurance Issues</th>
<th>Responsibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical question/test selection</td>
<td>Indications for immunohistochemistry; selection of stain(s)</td>
<td>Surgical pathologist; sometimes clinician</td>
</tr>
<tr>
<td>Specimen acquisition and management</td>
<td>Specimen collection, fixation processing, sectioning</td>
<td>Pathologist/technologist</td>
</tr>
<tr>
<td>Analytic issues</td>
<td>Qualifications of staff; intra- and interlaboratory proficiency testing of procedures</td>
<td>Pathologist/technologist</td>
</tr>
<tr>
<td>Results of validation and reporting</td>
<td>Criteria for positivity/negativity in relation to controls; content and organization of report; turnaround time</td>
<td>Pathologist/technologist</td>
</tr>
<tr>
<td>Interpretation, significance</td>
<td>Experience/qualifications of pathologist; proficiency testing of interpretational aspects; diagnostic, prognostic significance; appropriateness/correlation</td>
<td>Surgical pathologist and/or clinician</td>
</tr>
</tbody>
</table>

Reprinted with permission from Reference 47a.
Montone KT.  **Brigati DJ.**  Budgeon LR.
For 150 + yrs H&E

For 80 Yrs ImmFlu Frozen

For 40 + yrs of bad habits - IHC on FFPE

About 2000 - GOLD STANDARD DX was still H&E

THEN SUDDENLY 20 YRS AGO – new technology and EVERYTHING CHANGED

The first companion diagnostic was approved

Radiology went digital
PACS for handling huge files
Age of Digital Pathology begins
From 1975-- for 20 years IHC was used as ‘special stain’ for lineage related markers - keratin, CD45 etc.

Then in 1990s - BIOMARKERS
Prognostic and Predictive markers

The QUESTION changed–
NOT just is it there?

BUT how much is there?
Score, count, quantify????

Estrogen receptor - ER
Established ImmunoPx for Prognostic markers

Louis P Pertschuk

Jules Elias

"The testing has to be done right, and it’s not."

Craig Allred
1998. HercepTest – included Cell lines as ‘RUN’ controls
To assure greater consistency
- in labs
- and among labs

Bioengineered Cell lines. PDL-1 by IHC
High expressor
- medium
- neg

Dako

Courtesy Farah Patell-Socha
Horizon Discovery, Cambridge, UK.
IMPROVE RIGOUR OF EXISTING CONTROLS.
The Control series: how to optimize use of current controls

Recommendations from the International Ad Hoc Committee
Torlakovics, Cheung, Taylor et al.

**Standardization of Negative Controls in Dx IHC**

**Standardization of Positive Controls**

**Evolution of Quality Assurance of Clinical Immunohistochemistry in the Era of Precision Medicine**


When we have done all that – the method is the best we can make it --
There still is one remaining problem -- US!!
- the lack of objectivity of the pathologist

“Invented” the ‘old pathologist’

Inventing the ‘new pathologist’

The Digital Pathologist
PD-L1 IHC
Score -0%, 1%, 5%, 50% ?
Which negative?
Which positive?
Which to be treated?
What about Immune cells?
Can we all get same answer?
What is the ‘score’?
**PD-L1**  **Threshold** - 5%
Does the patient get treated or not?

How many total cancer cells
**Denominator**?

Impossible to count –
-so ‘guess’ half are cancer cells?
- If 600 in total
-- then about 300 cancer cells

How many positive Ca cells?
**Numerator**?

**Score % = positive cell count**
300

PDL-1 membrane stain
count the positive cancer cells
15 cells= 5 % threshold

**14** -no treatment
**15** -- $100,000 Rx

**TO SCORE ONE HIGH POWER X40 FIELD**

Percentage positive = **numerator:** +ve cancer cells
**denominator:** total ca cells
With digital help --- Multiplex IHC possible and ImmFlu makes a come back

- Detect BIOMARKER expression
- Achieve better cell ID Immune cell phenotyping
- Quantification = counting Accurate scoring
- Quantification = amount comparing intensity versus internal standard

Courtesy - Cliff Hoyt PerkinElmer, 2015
WITH DIGITAL and Artificial Intelligence HELP ----
What NEW THINGS are possible?
-- ‘rehabilitate’ fluorescence by restoring morphology – virtual H&E

Courtesy - Cliff Hoyt
PerkinElmer, 2015
Obstacles to digital pathology

RESOLUTION

SCANNING (acquisition, display) SPEED

IMAGE (file) STORAGE / SHARING /VIEWING

Apps for scoring (counting), quantification, analysis, metrics

Acceptance by pathologists

HARDWARE COSTS – falling <50K
SOFTWARE costs- access- CLOUD

REGULATORY and REIMBURSEMENT
Whole Slide Imaging Versus Microscopy for Primary Diagnosis in Surgical Pathology
A Multicenter Blinded Randomized Noninferiority Study of 1992 Cases (Pivotal Study)

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Technology marches on - THE TREND - is to “cloud based”

No special software; any hardware you like

All you need is

THE NET

a BROWSER

And a high res screen

Like Google Maps

Where are the nearest restaurants?

Where are the nearest Cancer cells?

Detecting Cancer Metastases on Gigapixel Pathology Images

Yun Liu et al. Martin C. Stumpe. GoogleBlog 2017
Milestones in Immunohistology

Histoology

Immunohistology

Immunology

Past

Future

Confused

Unclear

Perplexed

Disoriented

Bewildered

Prediction is difficult, especially about the future

Niels Bohr, 1885-1962

Clive R Taylor MD. PhD