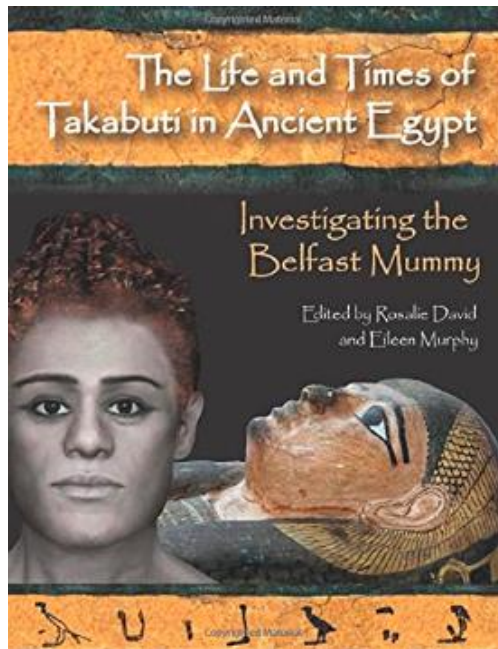


# Professor Anthony Freemont

BSc, MB.BS, MD, MRCS, MRCP, MRCPPath,  
FRCP, FRCP(E), FRCPath

Emeritus Professor of Pathology  
University of Manchester

(Procter Professor of Pathology, Professor of  
Osteoarticular Pathology, Professor of Biomedical  
Egyptology, on retirement in 2021)



**MITOCHONDRIAL DNA  
OF TAKABUTI  
DR K DROU & PROF A  
FREEMONT.**

**TAKABUTI'S HEALTH:  
PROTEOMICS – PROF A  
FREEMONT**

# scientific reports



OPEN

## The first reported case of the rare mitochondrial haplotype H4a1 in ancient Egypt

Konstantina Drosou<sup>1,2,✉</sup>, Thomas C. Collin<sup>3</sup>, Peter J. Freeman<sup>4</sup>, Robert Loynes<sup>1</sup> & Tony Freemont<sup>1</sup>

Takabuti, was a female who lived in ancient Egypt during the 25th Dynasty, c.660 BCE. Her mummified remains were brought to Belfast, Northern Ireland, in 1834 and are currently displayed in the Ulster Museum. To gain insight into Takabuti's ancestry, we used deep sampling of vertebral



Contents lists available at ScienceDirect

Journal of Archaeological Science: Reports

journal homepage: [www.elsevier.com/locate/jasrep](http://www.elsevier.com/locate/jasrep)



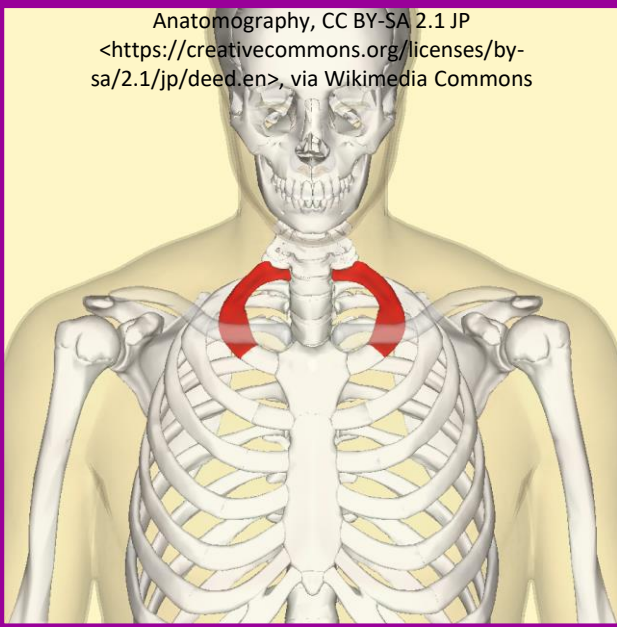
Enhancing mummy 'palaeobiographies' through the use of multidisciplinary techniques and approaches

K.N. White<sup>a,b,\*</sup>, D. Chiasserini<sup>c</sup>, R. Loynes<sup>a</sup>, A.R. David<sup>a</sup>, B.E. van Dongen<sup>b</sup>, K. Drosou<sup>a</sup>, R. Forshaw<sup>a</sup>, S. Fraser<sup>b</sup>, P. Causey-Freeman<sup>d</sup>, J. Metcalfe<sup>a</sup>, E. Murphy<sup>e</sup>, M. Regan<sup>f,h</sup>, P.J. Reimer<sup>e</sup>, D.G. Tosh<sup>h</sup>, A. Whetton<sup>g</sup>, A.J. Freemont<sup>a</sup>

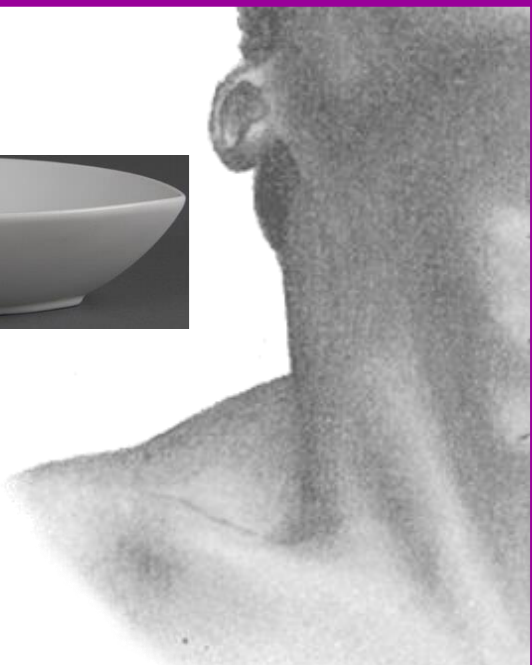
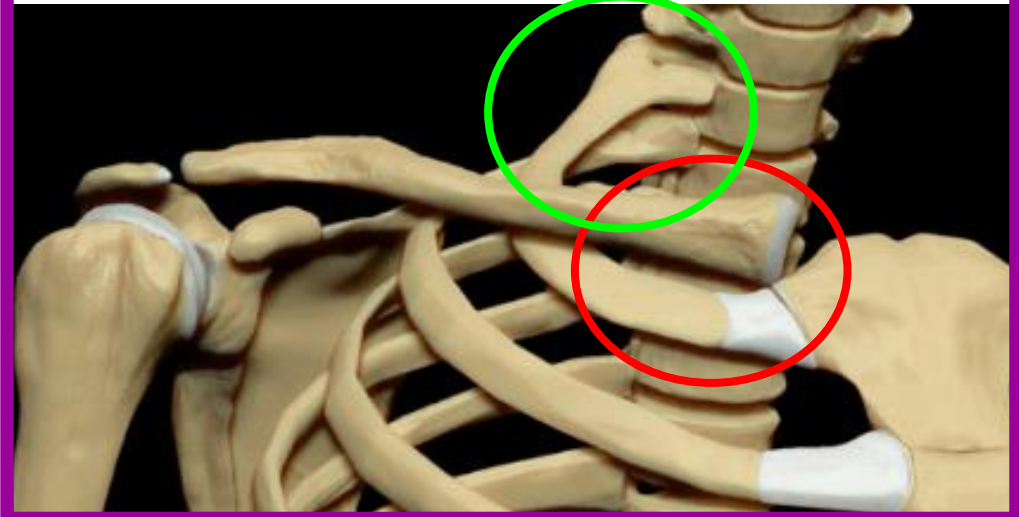
<sup>a</sup> The KNH Centre for Biomedical Egyptology, University of Manchester, UK  
<sup>b</sup> Department of Earth and Environmental Sciences, School of Natural Sciences, University of Manchester, UK  
<sup>c</sup> The Stoller Biomarker Discovery Centre, University of Manchester, UK  
<sup>d</sup> Informatics, Imaging and Data Sciences, University of Manchester, UK  
<sup>e</sup> Archaeology and Palaeoecology, School of Natural and Built Environment, Queen's University Belfast, UK  
<sup>f</sup> Kingsbridge Private Hospital, Belfast, UK  
<sup>g</sup> School of Veterinary Medicine University of Surrey, Guildford, UK  
<sup>h</sup> National Museums Northern Ireland, Belfast, UK

Initial report on Mr Bayoh's  
**isolated**  
left  
**1<sup>st</sup> rib**  
fracture

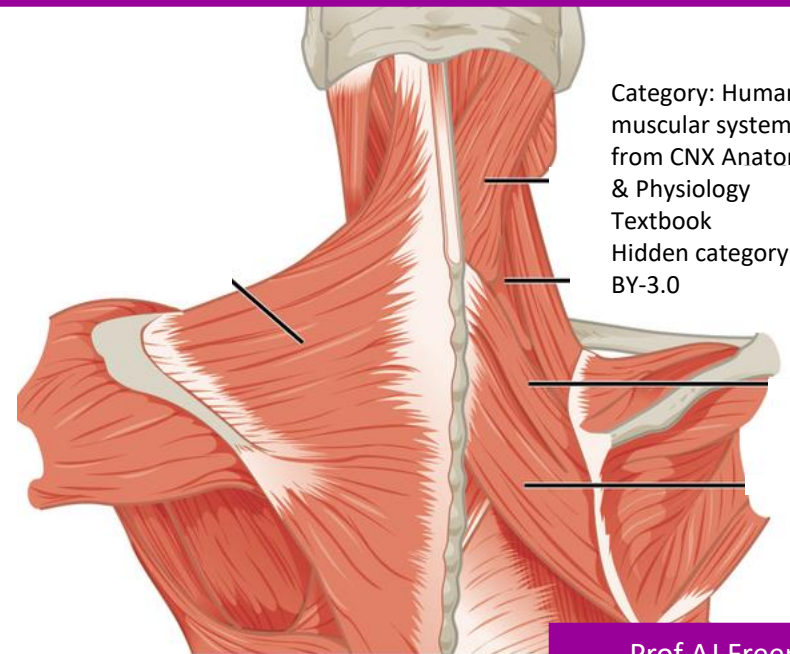
Anatomography, CC BY-SA 2.1 JP  
<<https://creativecommons.org/licenses/by-sa/2.1/jp/deed.en>>, via Wikimedia Commons



By DrJanaOfficial - Own work, CC BY-SA 4.0,  
[https://commons.wikimedia.org/wiki/File:Clavicle\\_3d\\_Model.gif](https://commons.wikimedia.org/wiki/File:Clavicle_3d_Model.gif)



Henry Gray (1918)  
Anatomy of the  
Human Body 20<sup>th</sup>  
edition  
Henry Vandyke  
Carter (1831–  
1897)



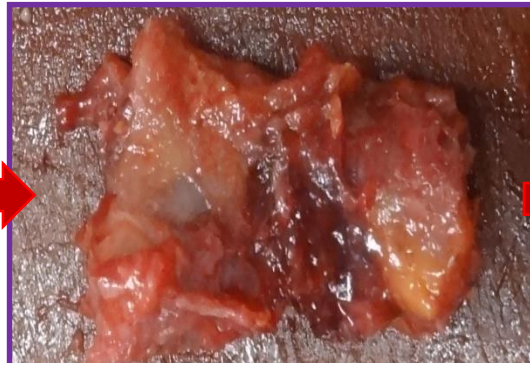
Category: Human  
muscular system  
from CNX Anatomy  
& Physiology  
Textbook  
Hidden category: CC-  
BY-3.0

Prof AJ Freemont  
University of Manchester

On 27<sup>th</sup> April 2017 I received 6 microscope slides, stained H&E and Perl's. I reviewed the slides and noted:

- Part of a fractured bone.
- Tissue decomposition.
- Appearances suspicious of bleeding into the fracture and adjacent bone marrow/soft tissues.
- Osteocyte necrosis in bone adjacent to the fracture line.

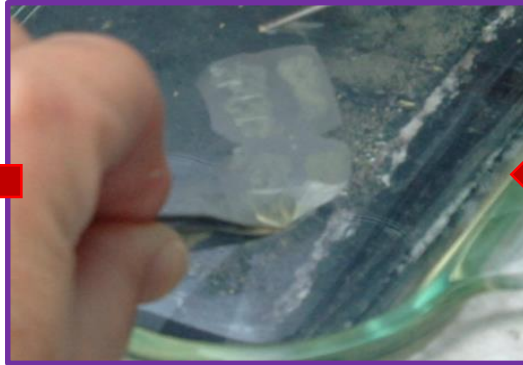
# Making a microscope slide



Tissue block

Flickr images uploaded by Flickr upload botCC-BY-SA-2.0Files from Ed Uthman Flickr stream

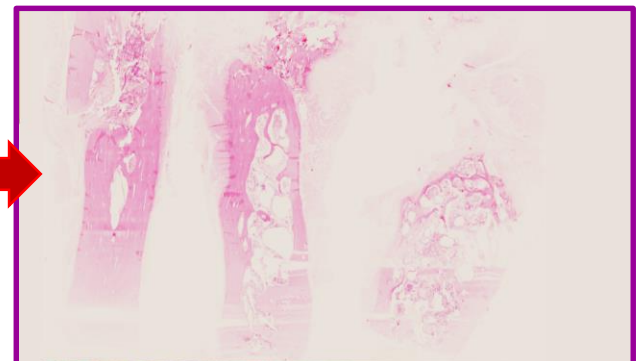
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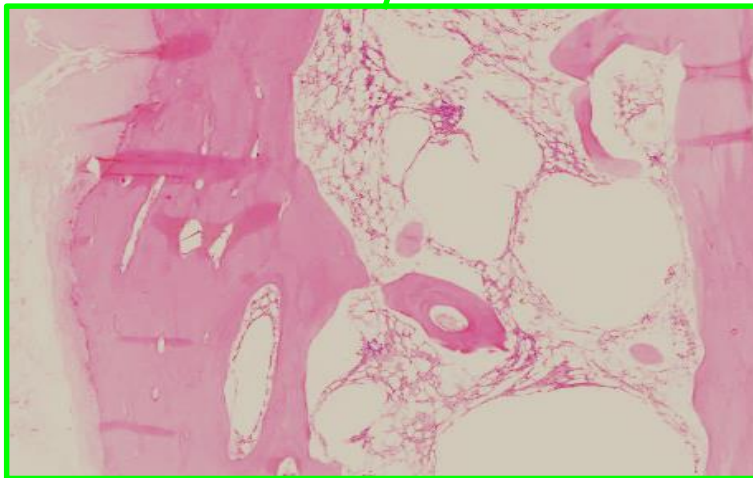
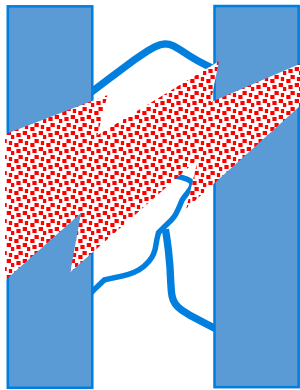
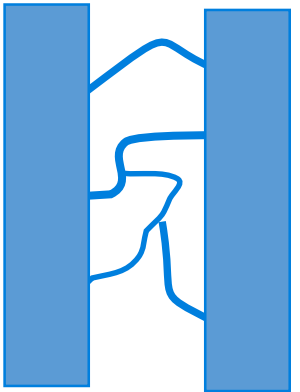
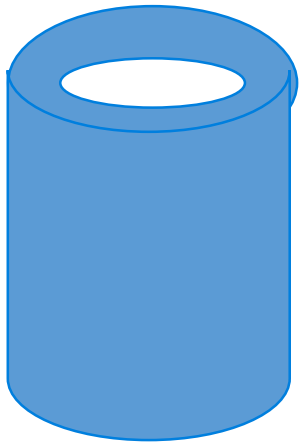


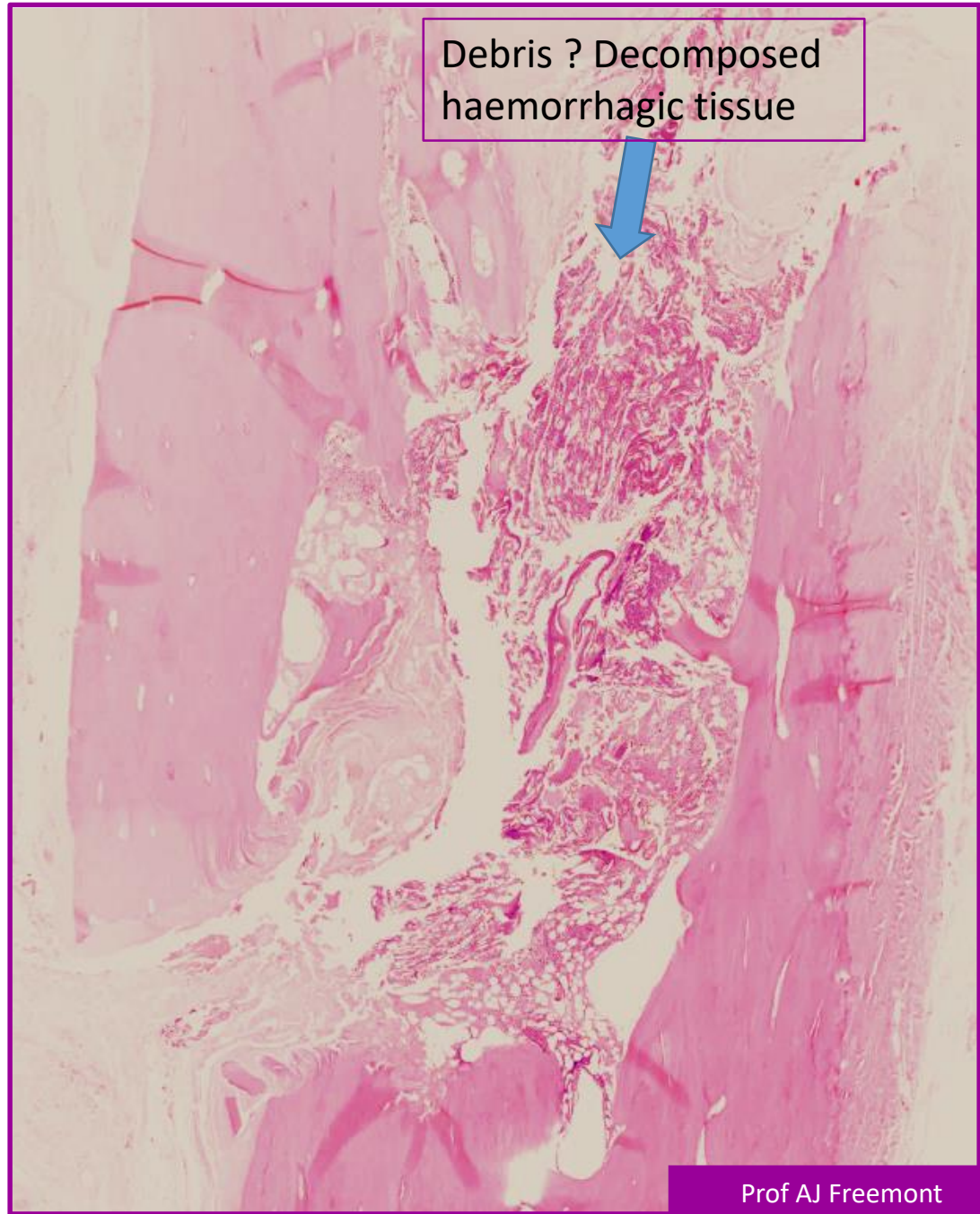
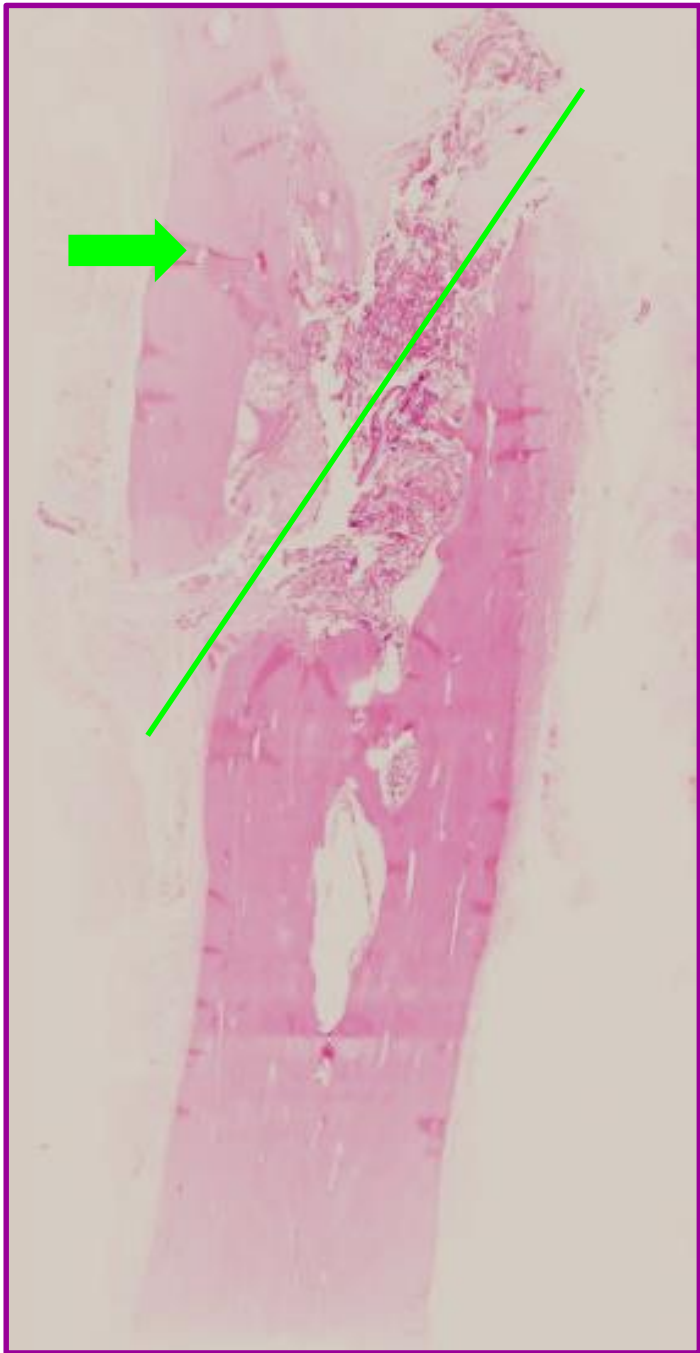
Staining



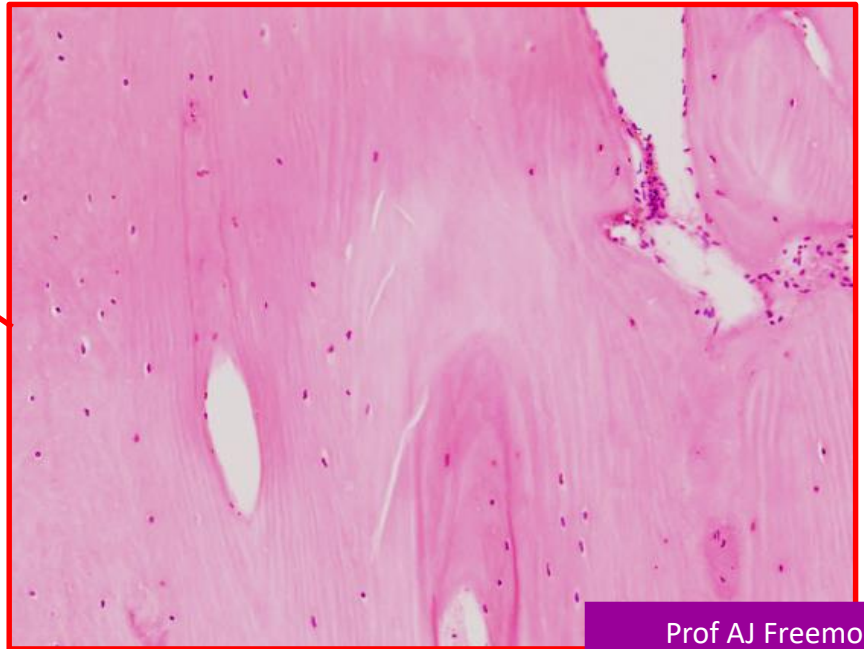
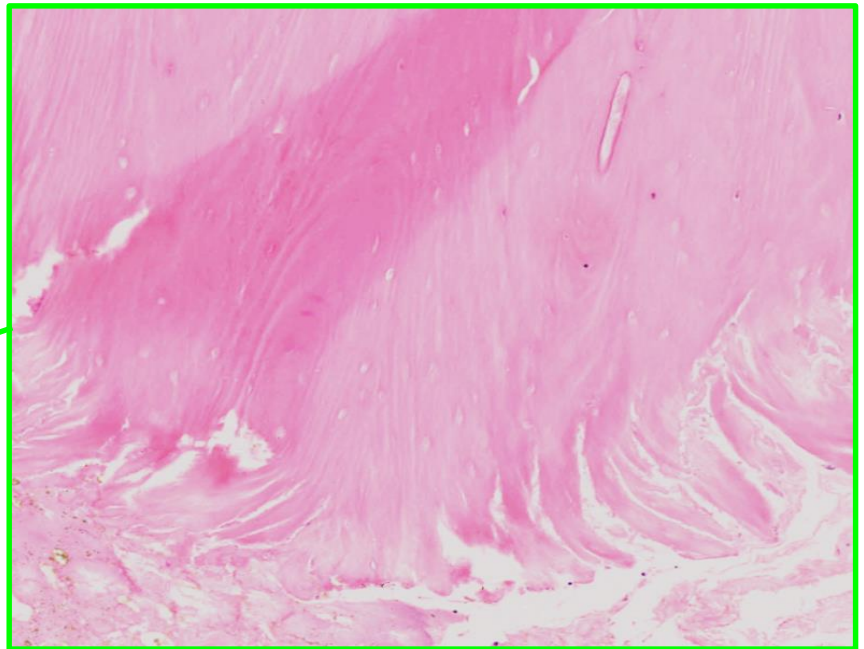
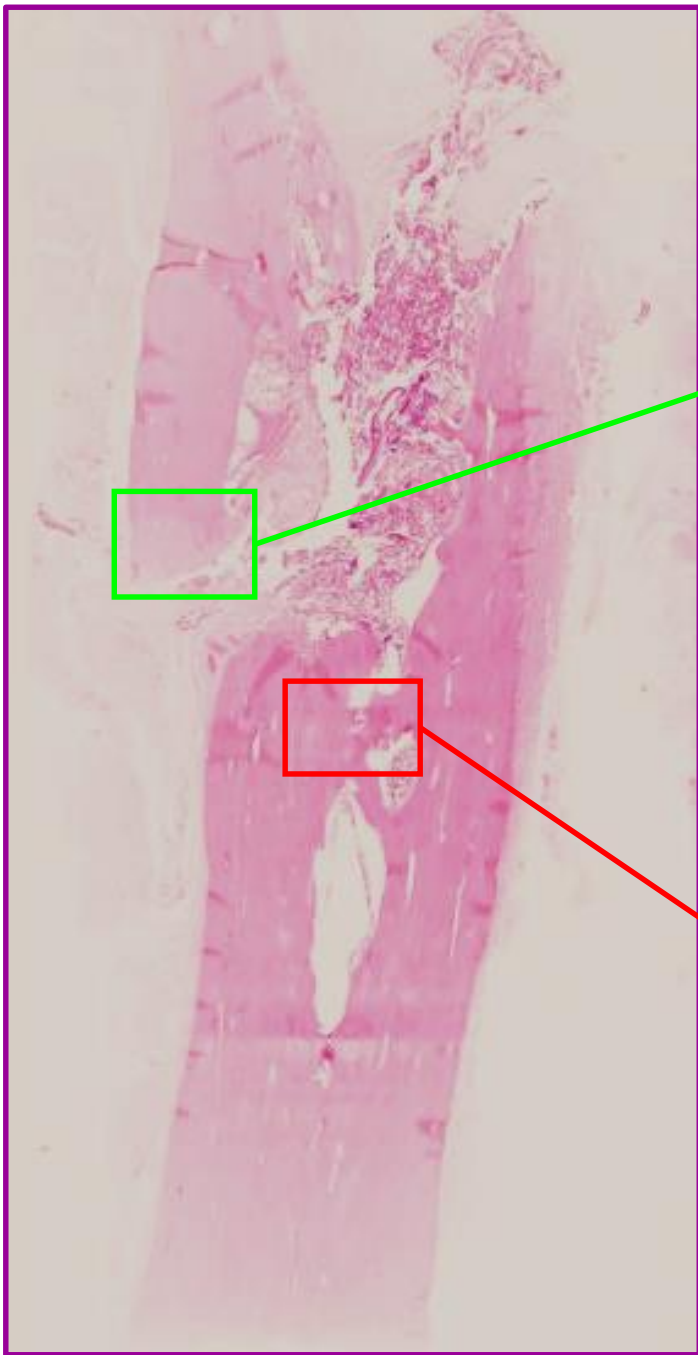
Received 6 slides made from 1 block 3 stained H&E & 3 Perls

Prof AJ Freemont  
University of Manchester









Is fracture present? ✓

Why decomposition present? ?

How old is fracture?

- Osteocyte Necrosis (in life) ✓  
( >2 hours before death)
- Bleeding (Haemorrhage) ? - Decomposition
  - Present (amount/distribution) ?
  - ? Fibrin (>6 hours before death) ?
- Evidence of bone healing X

# Subsequent studies in 2017

Why decomposition present?

Components of haemorrhage?

- Haemorrhage present – Red blood cells
- Fibrin allows aging – visible >6hours old



**Special Stains – Identifying molecular structure using colour chemistry on extra sections**

# Why was decomposition present?

- Initially no fracture on x-rays/autopsy
- Sensitive imaging: isolated 1<sup>st</sup> rib fracture
- Further autopsy 25 days after the first
- Local tissue decomposition had started
- Not unusual in refrigerated bodies

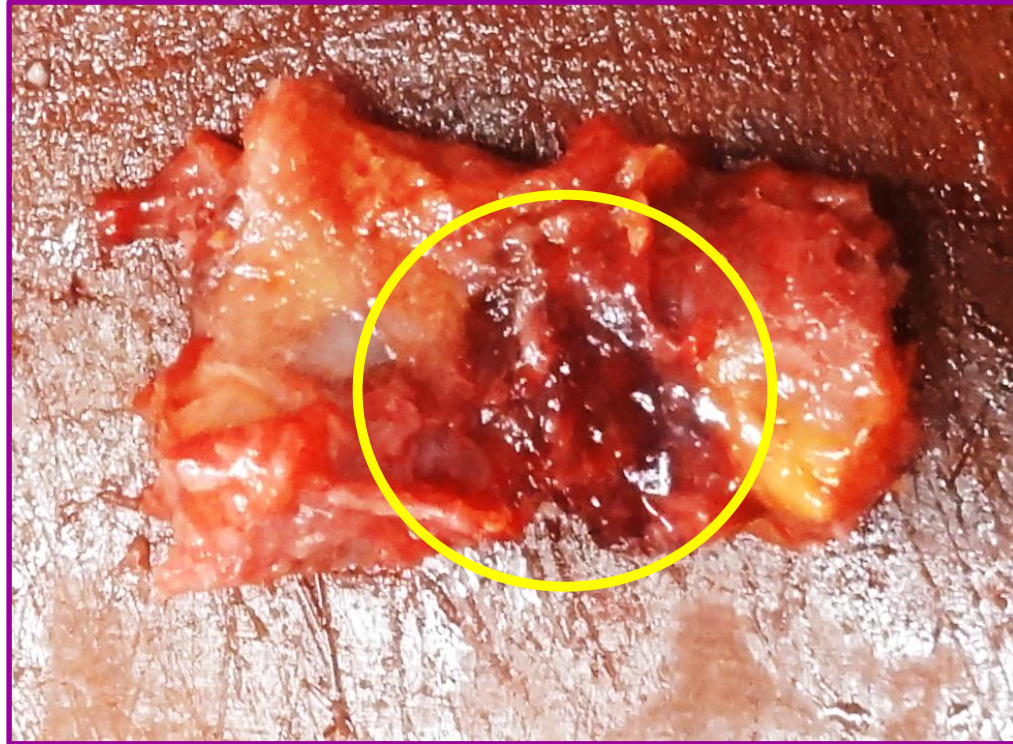
## **Nature of any haemorrhage?**

- Red blood cells (number/distribution)
- Fibrin (also aging)

## **Nature of debris in fracture line?**

## **Fungi of decomposition present?**

# Sent images of fractures site

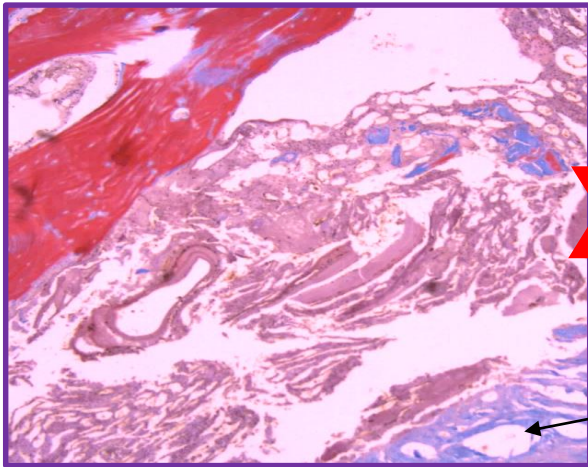


Area circled: Dark red, supporting possible ante mortem haemorrhage

Red blood cells doughnut shaped balloons.  
Surface of the “balloon” – Glycophorin A (GlyA)  
Decomposition: balloon bursts - surface fragments  
Recognise GlyA using brown stain (IHC)



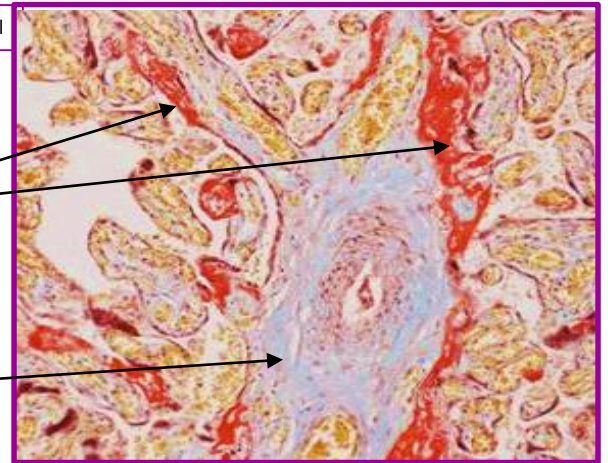
<http://www.histologicaltechniques.com/MSB.html>



**MSB**

Visible fibrin  
Orange-red

Collagen blue

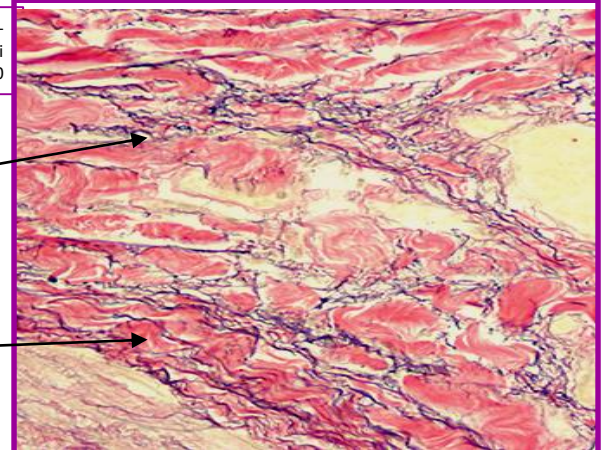


[https://histolab.e-line.nu/en/nya\\_e-line/Chemicals/Farglosningar\\_och\\_pulver/Elastic\\_Van\\_Gieson\\_Stain\\_Kit\\_%28Miller%29\\_%28EVG%29\\_500\\_test?id=AS-RRSK11-500](https://histolab.e-line.nu/en/nya_e-line/Chemicals/Farglosningar_och_pulver/Elastic_Van_Gieson_Stain_Kit_%28Miller%29_%28EVG%29_500_test?id=AS-RRSK11-500)

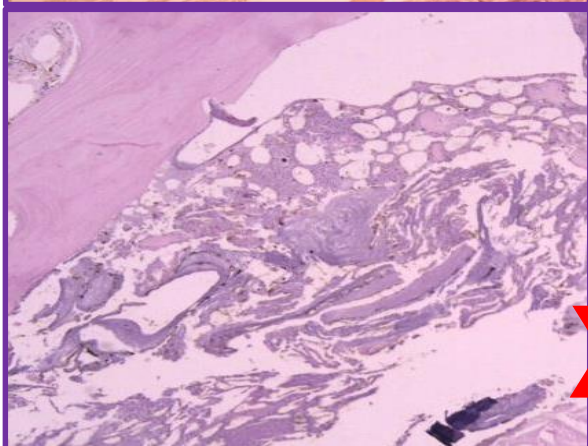


**Elastin  
EVG**

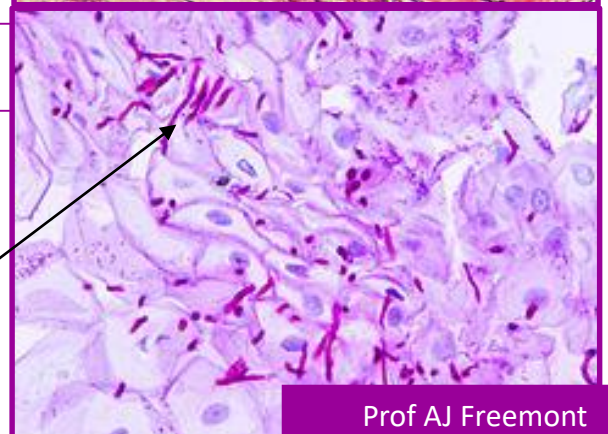
Collagen  
brick red



• [License migration redundant GFDL CC-BY-SA-3.0-migrated](#)  
• [https://commons.wikimedia.org/wiki/File:Esophageal\\_candidiasis\\_\(2\)\\_PAS\\_stain.jpg](https://commons.wikimedia.org/wiki/File:Esophageal_candidiasis_(2)_PAS_stain.jpg)



**PAS**  
Fungi  
Purple-Magenta





# My view of fracture in 2017

- Solitary left first rib fracture
- Fracture occurred in life

Amount/distribution of bleeding [GlyA]

Presence of osteocyte necrosis

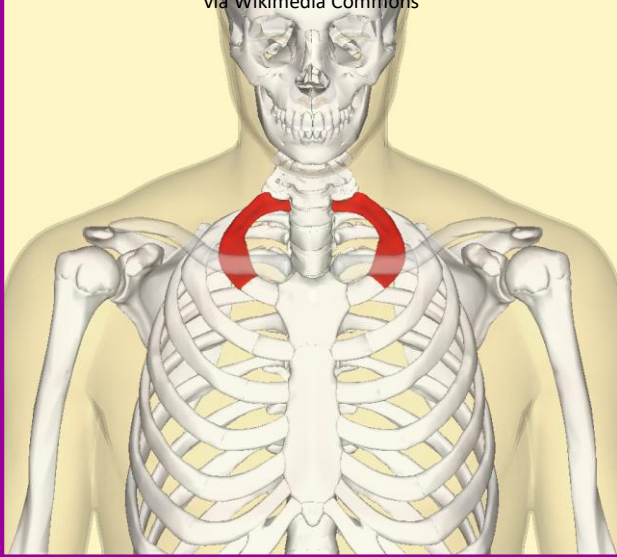
- Fracture probably occurred between 2 and 6 hours before death.

>2 hours: osteocyte necrosis

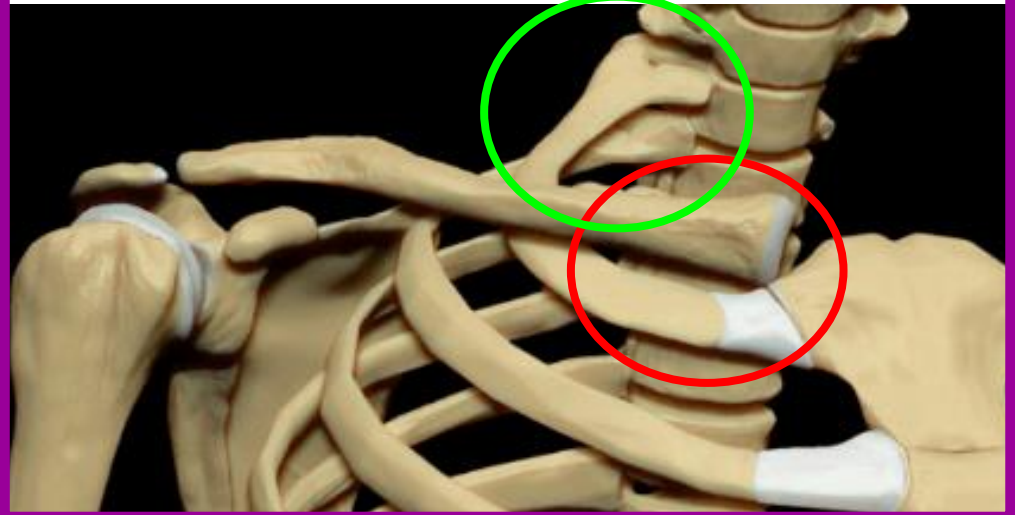
<6 hours: no visible fibrin

Cause of an isolated first  
rib fracture generally  
and in this case

Anatomography, CC BY-SA 2.1 JP  
<<https://creativecommons.org/licenses/by-sa/2.1/jp/deed.en>>  
via Wikimedia Commons



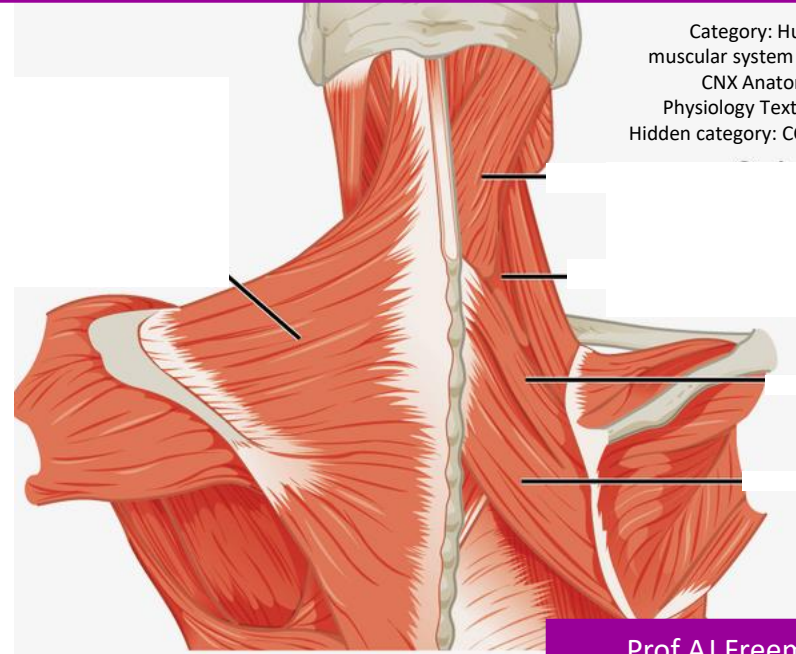
By DrJanaOfficial - Own work, CC BY-SA 4.0,  
[https://commons.wikimedia.org/wiki/File:Clavicle\\_3d\\_Model.gif](https://commons.wikimedia.org/wiki/File:Clavicle_3d_Model.gif)



Henry Gray (1918)  
Anatomy of the Human  
Body 20<sup>th</sup> edition  
Henry Vandyke Carter  
(1831–1897)



Category: Human  
muscular system from  
CNX Anatomy &  
Physiology Textbook  
Hidden category: CC-BY-  
3.0



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# Causes of isolated 1<sup>st</sup> rib fracture (either side)

Indicative site of fracture

Scalene tubercle

Anatomical drawing of first rib

Fig. 200, p. 184, Morris' human anatomy : a complete systematic treatise, 9th ed.(1933)

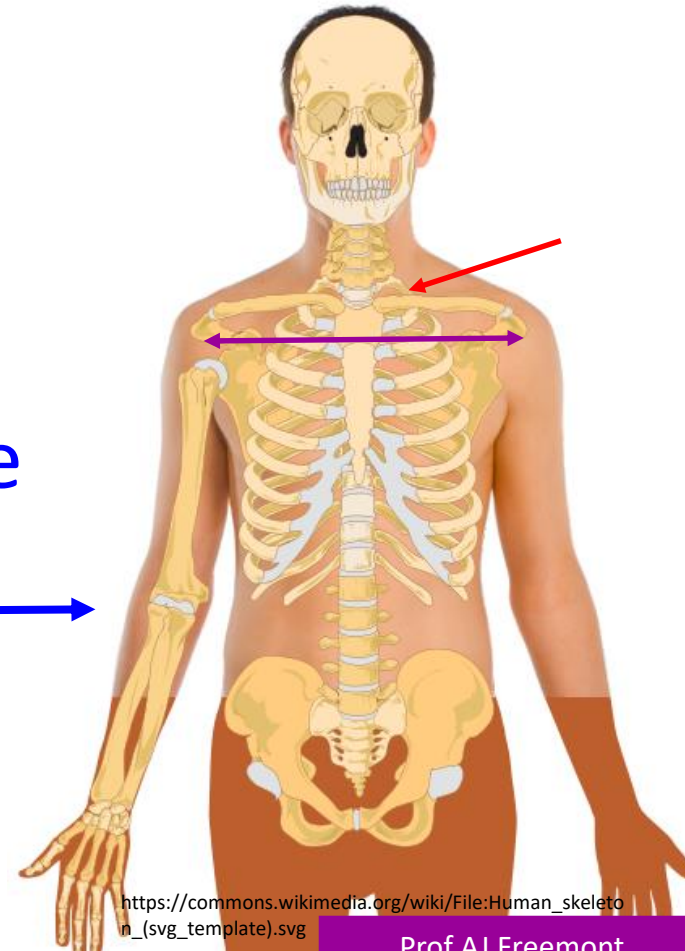
- Direct external trauma (e.g. a kick directly to the rib)
- Indirect trauma e.g.:
  - falling on an outstretched arm
  - blow to the shoulder
  - Fracture caused by violent muscular contraction (Almost restricted to green area on diagram)

**Based on witness statements available at time, my knowledge and understanding, and my findings:**

- Direct external trauma – **Unlikely**  
Isolated fracture, no reported event
- Falling on outstretched arm (or equivalent) – **Likely**  
Altercation with friend and interactions with police
- Blow to the shoulder (or equivalent) – **Possible**  
? fallen/been brought down onto shoulder
- Violent muscular contraction – **Unlikely**  
Press up + heavy weight on body: fracture site  
inconsistent

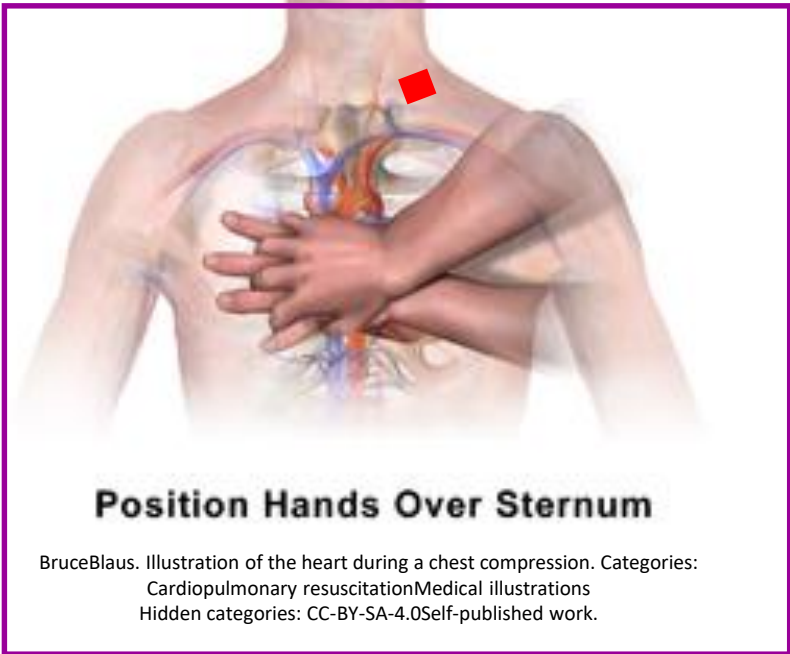
# Other hypothesised causes

- Handcuffs - **Unlikely**  
Forces inconsistent
- Fight - **Possible**  
Fall or equivalent
- Press up – **Unlikely**  
Fracture in wrong site
- Squeeze – **Unlikely** →
- CPR - **Unlikely**  
See next slide

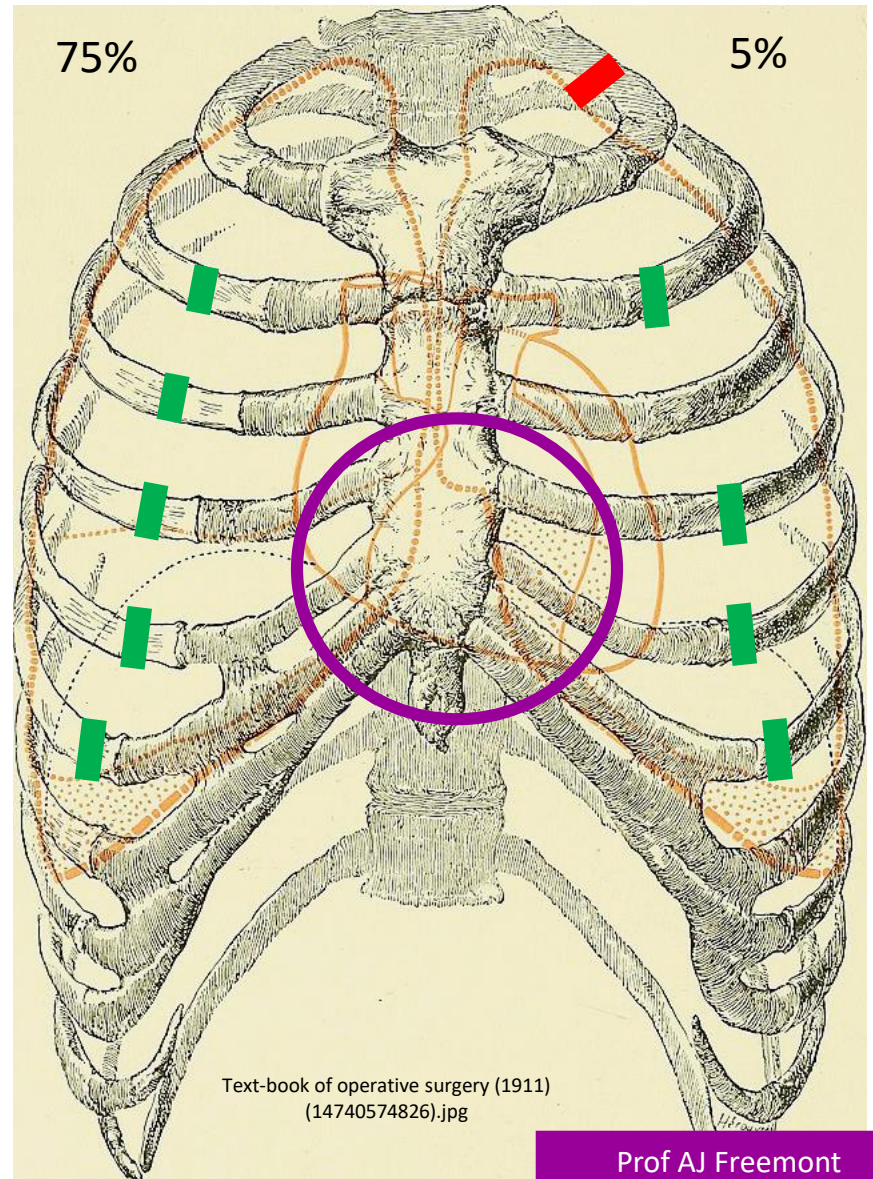


[https://commons.wikimedia.org/wiki/File:Human\\_skeleton\\_\(svg\\_template\).svg](https://commons.wikimedia.org/wiki/File:Human_skeleton_(svg_template).svg)

# COULD ISOLATED FRACTURE OF THE 1<sup>ST</sup> RIB BE CAUSED BY CPR?



Video by Bangkok Hospital Phuket. Mikael Häggström (talk | contribs)Category: Cardiopulmonary resuscitation  
Hidden categories: CC-BY-3.0Animated GIF files



# New data have provided new insights

- Review of new scientific data from 2017
- New information from Inquiry team



- **Clearer timeline of events.**
  - What happened and when in the 6-12 hours before death
  - Management of collapse and arrest.
- **Toxicology analysis**
- **More evidence on aging fractures**
  - Published data on aging fractures
  - Osteocyte apoptosis biology

# Events in the 6-12 hours before death

Where and how fracture might have occurred

Altercation with friend or police

Greater understanding of collapse and cardiac arrest

- When heart stopped pumping
- No cardiac arrest until hospital
- Hospital CPR: systolic pressure: 70-140mmHg

**Time of death - 09.04 3/5/15**

# Toxicology analysis

## **Psychostimulants:** MDMA and $\alpha$ PVP

- Altered pain perception
- Behaviour changing

## **Synthetic Androgen:** Nandrolone or similar

- Direct effect on osteocyte apoptosis
- May change time closest to death that apoptosis might 1<sup>st</sup> be seen

# More evidence on aging fractures?

- Published data on aging fractures



Osteocyte apoptosis: 1 hr before death in infants

Unpublished: Adults: 2hrs. Too few cases for publication

- Osteocyte apoptosis biology  
Discussed in toxicology

## My view of fracture in 2023

- Solitary left first rib fracture
- Occurred in life
- Occurred <6 hours before death (09.04)
- Nandrolone effects & data from infants - could have occurred <2hrs before death
- Probably occurred in altercation with friend/police: ~2.5/1.75hr before death
- Caused by indirect injury: favour fall onto outstretched arm or equivalent