The Mysterious case of the positive Kleihauer result

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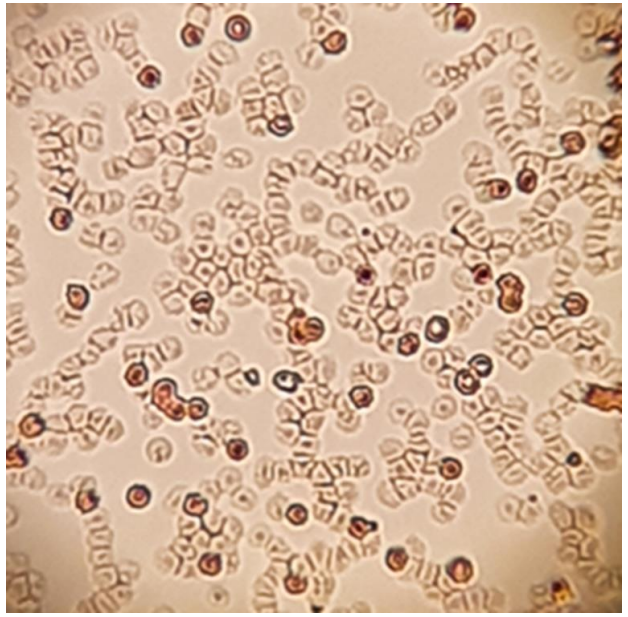
Background

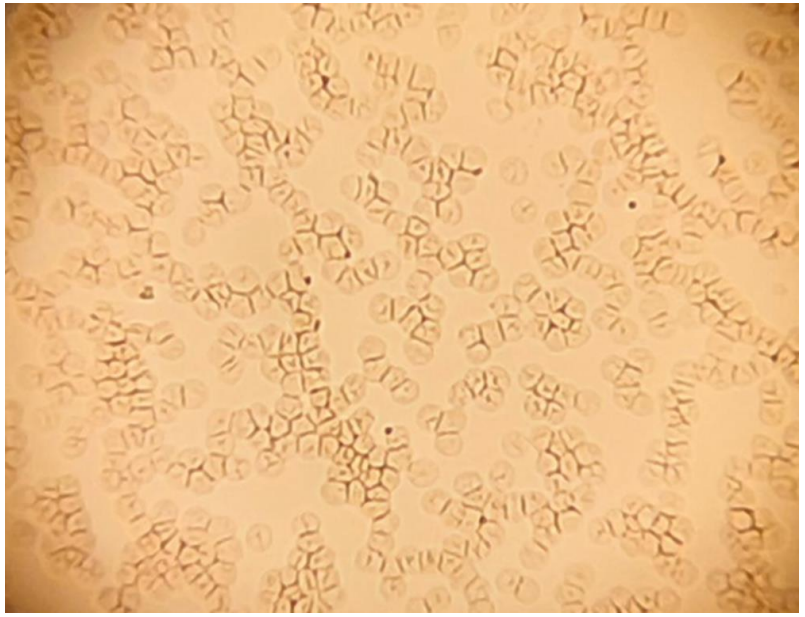
- Rh D Female patient – post delivery
- Sample received in lab for Group and Kleihauer
- Baby predicted RhD Positive by ffDNA
- However a sample was sent to the lab and tested
- Baby group was O RhD positive
- Use Clintech kit for acid elution screening (Kleihauer)
- Positive results sent to NHSBT for Flow cytometry

Kleihauer Controls

POSITIVE

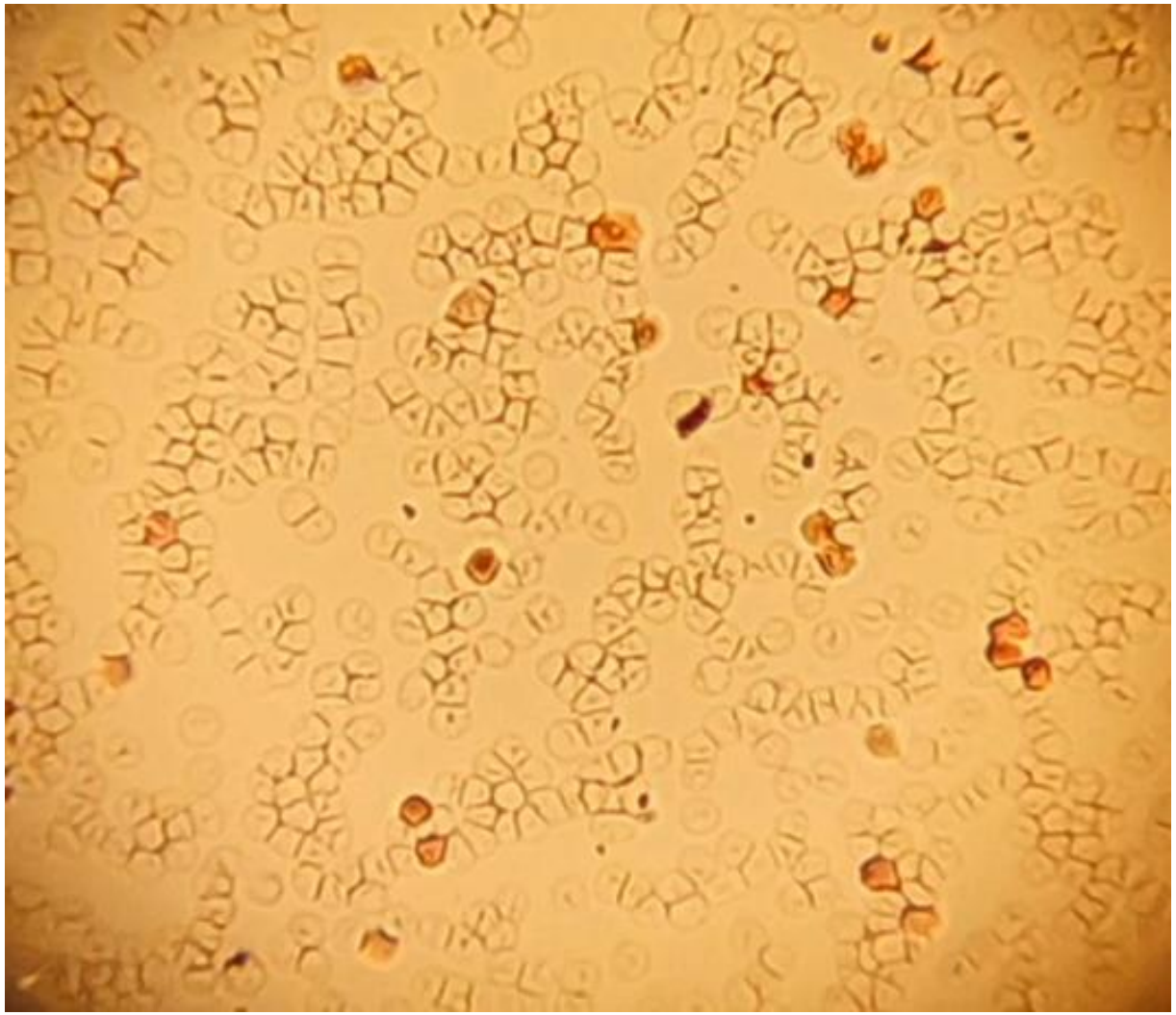


NEGATIVE



Patient's Kleihauer result

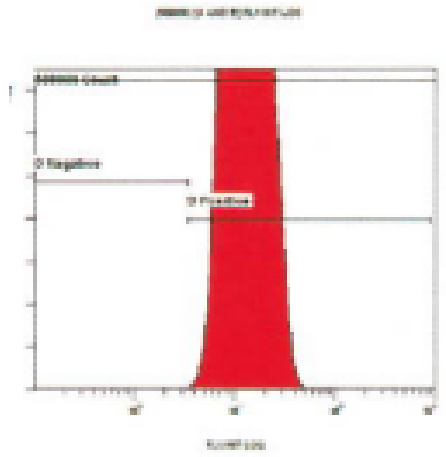
- Positive
- Films repeated to check for contamination
- Again positive
- Sample sent to NHSBT for Flow cytometry
- Standard dose of prophylactic anti-D issued (1500iu) whilst we waited on flow results



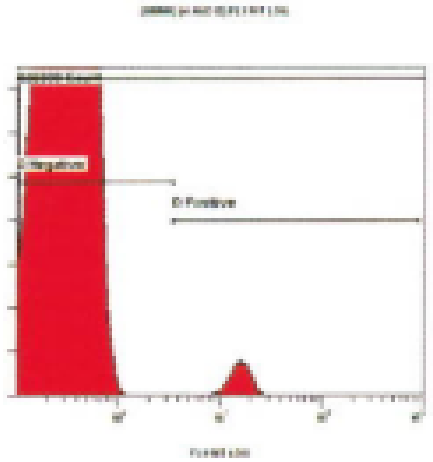
Flow cytometry results

- Flow results came back as <1mL bleed
- This had us confused as the film was very positive
- Due to discrepancy, requested NHSBT lab to check results
- Sample was rerun and produced the same results – no RhD positive cells

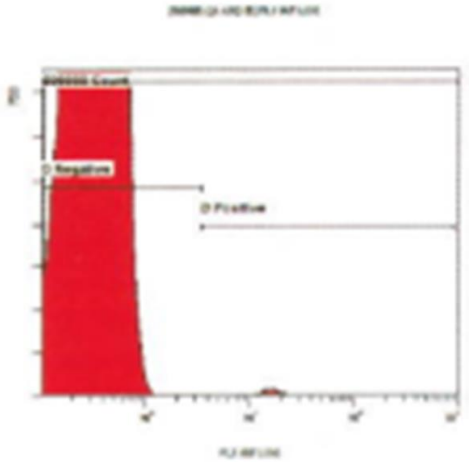
FL1 plots for the controls



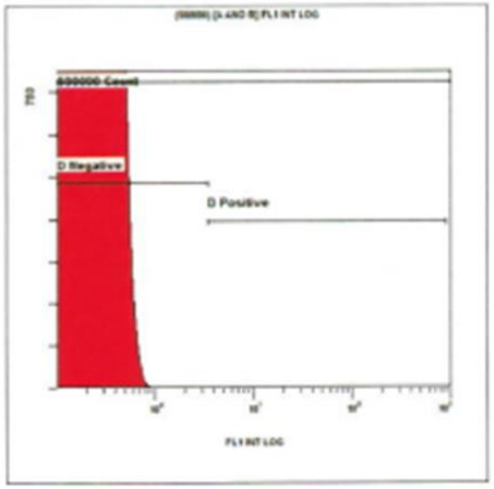
100% control



1% control



0.2% control



(500000) [A AND B] FL1 INT LOG

Region	Number	%Total	%Gated
ALL	500000	99.86	100.00
500000 Count	500000	99.86	100.00
D Negative	473335	94.53	94.67
D Positive	68	0.01	0.01

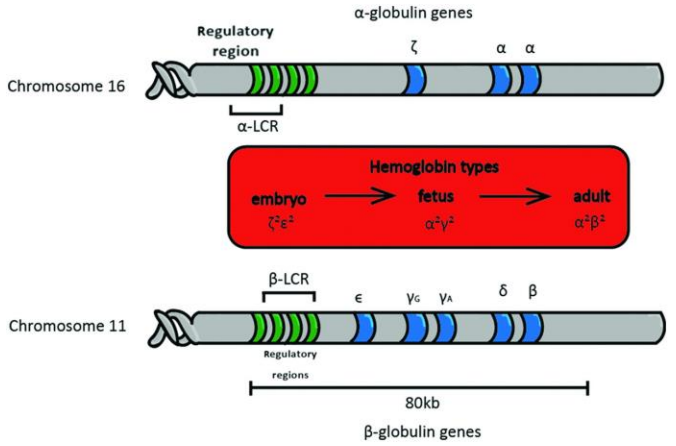
Our patient

What is the cause of the positive Kleihauer films?

- Are these the mother cells with raised HbF?
- Are these baby cells with a D variant?

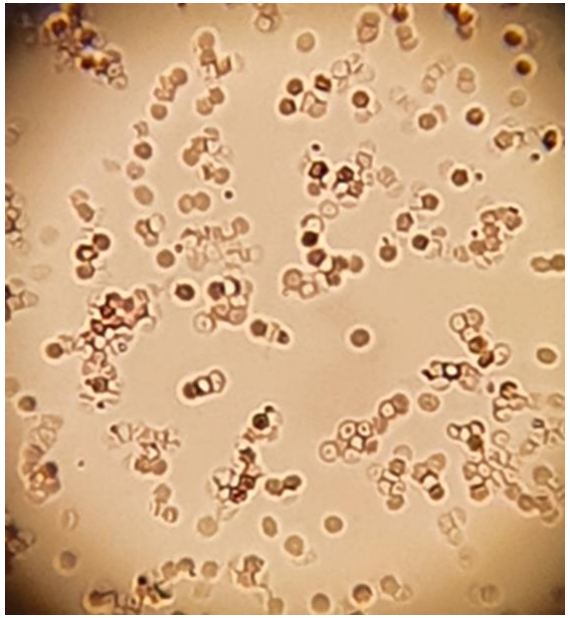
Haemoglobinopathy evaluation

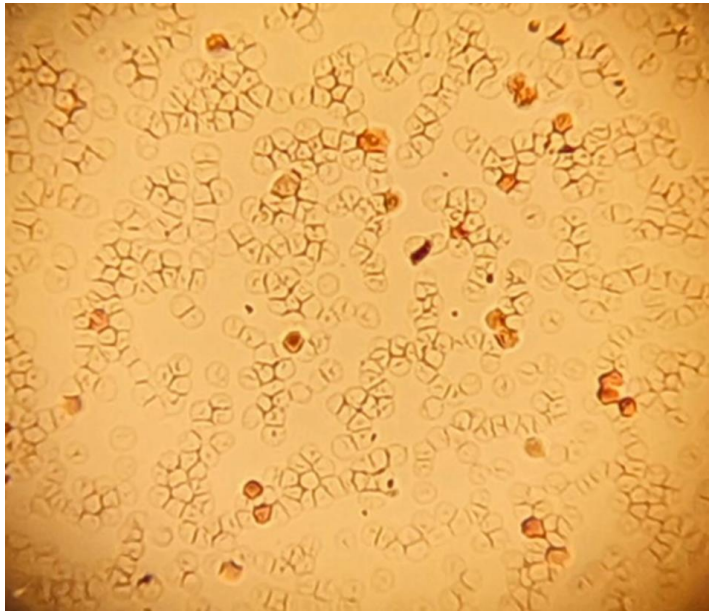
- Booking Haemoglobinopathy done at 11 weeks +6
- HbA2 = 5.1%
- HbF = 2.9%
- Patient is a beta-thalassaemia carrier



HbF Kleihauer films

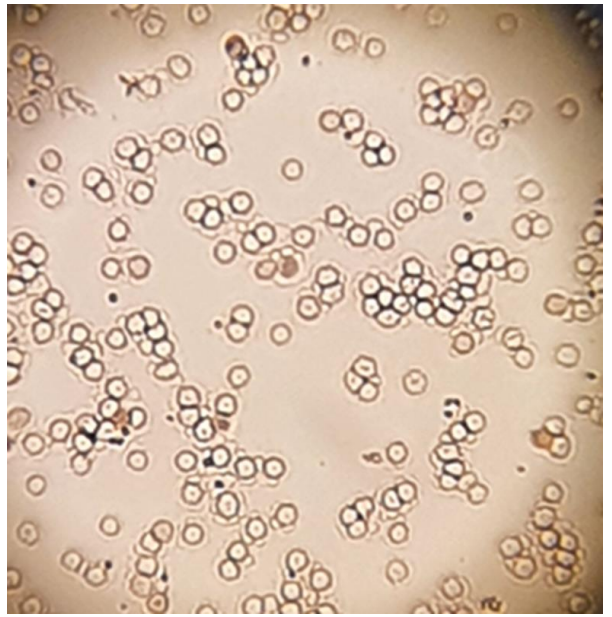
RAISED HBF 10.6%





Our patient HbF 2.9%

BOOKING BLOODS WITH HBF 3.5%



So what next?

- After discussion with NHSBT it was suggested we perform a manual count
- We don't normally estimate due to the huge variation in results – this is why we send off for flow
- Both the site lead and senior BMS in BT have the most experience in Kleihauer quantifications so both performed a count

- The FMH was calculated as 109mL bleed

The decision made

- After discussion with our haematology consultants and NHSBT it was decided to give the patient a dose of prophylactic anti-D big enough to cover the calculated bleed
- 8 vials (1500IU) via IV
- The patient received her anti-D and repeat sample requested for 48 hours later

We didn’t stop there!

- The baby sample was sent to NHSBT to check for a D variant

- On the repeat kleihauer sample, NHSBT requested that we retest the HbF

Results from the new sample

- Baby's group came back as RhD positive – no variant

- HbF on repeat kleihauer sample = 4.2%
- A little bit higher but still not enough to explain all the cells seen on the film

- Repeat Kleihauer film:
- If baby's cells, we would expect all the stained cells to have been removed due to anti-D prophylaxis
- If patients own RhD negative cells they would still be present

Kleihauer results from the new sample

- Kleihauer film revealed numerous RBC with a grey pinkish appearance, as expected to be seen with a raised HbF

CONCLUSION:

- The cells we were seeing must have been maternal cells and NOT foetal cells

Final thoughts

- With many, many years worth of experience between all BMS and consultants involved we acted in the best interest of the patient
- If it had been a case of the flow cytometry results being incorrect for any reason, then this could have lead to the patient potentially being sensitised and producing allo anti-D
- If time had not been a factor, we would have waited for all the investigation to be complete before giving anti-D prophylaxis
- We still don't have a definite answer – and all the information we have still doesn't quite add up
- We felt that the potential harm of not giving prophylactic anti-D out weighed the harm of giving it
- Later, a consultant at NHSBT indicated that we shouldn't have given the anti-D and should trust the flow results. Our response was that errors do happen and we acted in the best interest of the patient, questioning results that do not make sense
- If we had to do this all again would we do the same thing? Probably yes
- What would you do in this situation?

Thank-you!

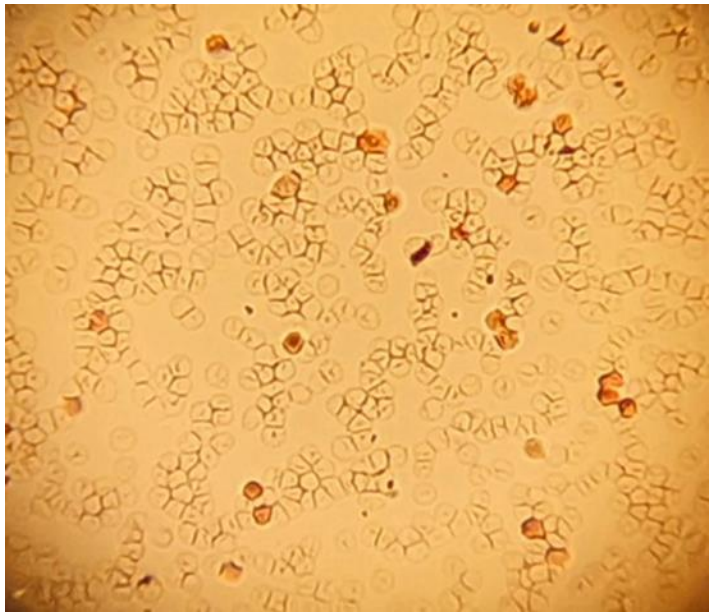
QUESTIONS?

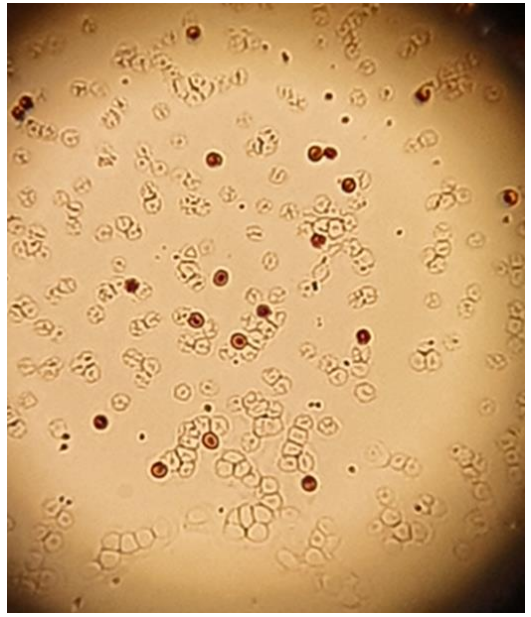
Additional information

Cell staining

BETA-THALASSEMIA PATIENT

POSITIVE CONTROL



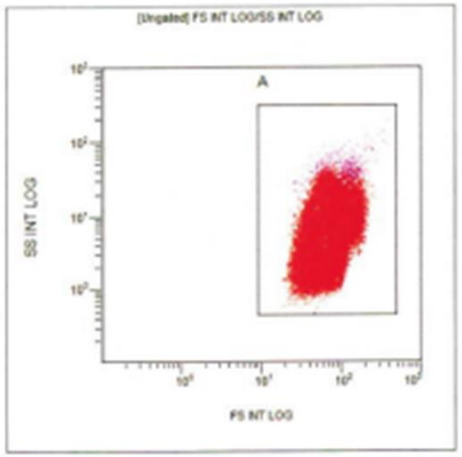


Our patient's Kleihauer result

Flow cytometry

FORWARD SCATTER VS SIDE SCATTER PLOT

Gate A is drawn around the blood cells

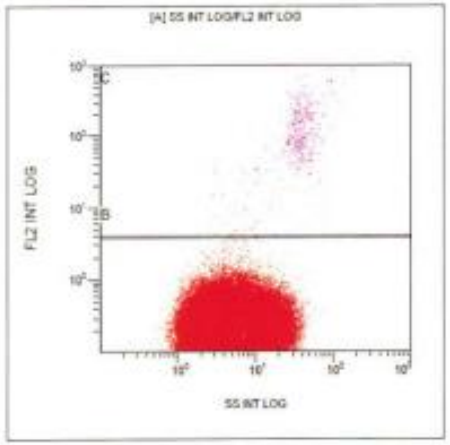


[Ungated] FS INT LOG/SS INT LOG

Region	Number	%Total	%Gated
ALL	500699	100.00	100.00
A	500698	100.00	100.00

SIDE SCATTER VS FL2 SCATTERPLOT

CD66b is a granulocyte marker, so the positive events in purple (Gate C) are granulocytes, the negative events in red (Gate B) are the red cells.



[A] SS INT LOG/FL2 INT LOG

Region	Number	%Total	%Gated
ALL	500698	100.00	100.00
B	500000	99.86	99.86
C	707	0.14	0.14