Higher Specialist Diploma

Haematology

Examination 2019

Paper 3

Discipline-specific questions

120 minutes

Attempt 3 out of 6 questions

Instructions to candidates

1. Record your candidate number, qualification title and where appropriate the discipline and examination paper number on the front sheet of the answer booklet

2. Record your candidate number and the page number in the spaces provided on the answer sheets

3. Begin each new answer on a new page

4. Write on one side of the answer sheet only

5. Each question is worth 100 marks

1. Discuss causes of leucopenia.

2. Critically discuss the procedures used for the differentiation of polycythemia vera and secondary erythrocytosis.

3. Critically discuss the detection and clinical significance of schistocytes in the peripheral blood smear.

4. With reference to the regulatory mechanism of iron storage, critically discuss methods used to investigate disruption of this mechanism.

5. Critically discuss techniques for detection of inhibitors to FVIII and methods for monitoring treatment of patients with FVIII inhibitors.

6. Critically evaluate currently available assays for quantifying von Willebrand factor function.

Higher Specialist Diploma

Haematology

Examination 2019

Paper 4

Case studies

120 minutes

Attempt all case studies

Instructions to candidates

1. Record your candidate number, qualification title and where appropriate the discipline and examination paper number on the front sheet of the answer booklet

2. Record your candidate number and the page number in the spaces provided on the answer sheets

3. Begin each new answer on a new page

4. Write on one side of the answer sheet only

5. **Each case study is worth 100 marks**

Seen case study

1.

A 29 year old West African woman was hospitalised in the 20th week of her first pregnancy for severe groin pain and left lower leg swelling. A Doppler ultrasound scan confirmed extensive venous thrombosis in her left leg. Her laboratory results at presentation are given below in Table 1.

A family history revealed that the patient's 59 year old father had experienced recurrent deep vein thrombosis in his mid-40s and was on long-term warfarin, although the underlying cause was never established. The patient's two sisters and one brother, all older, had no relevant history. The patient was put on low molecular weight heparin, which was maintained for the remainder of her pregnancy, during which she experienced no further thrombotic events. She had an uneventful, spontaneous vaginal delivery and the low molecular weight heparin was then discontinued.

Table 1: Coagulation screen and thrombophilia assays

Test/assay	Result	Units	Reference range
Prothrombin time	12.6	s	10.0 – 14.0
APTT (LA-insensitive)	32.1	s	32.0 – 42.0
Fibrinogen (Clauss)	4.9	g/L	2.0 – 4.0
Thrombin time	9.4	s	9.0 – 11.0
D-dimers	2.10	mg/L FEU	>0.55
Antithrombin activity (chromogenic; bovine FIIa)	79	iu/dL	82 - 118
Protein C activity (chromogenic)	145	iu/dL	75 - 140
Free protein S antigen	50	u/dL	Female 64 – 130 Male 80 - 140
APTT-based APCR assay (1+4 dilution in FV deficient plasma)	2.82	Ratio	2.45 – 3.52
dRVVT screening test	0.95	Ratio	0.85 – 1.15
LA-sensitive APTT screening test	0.82	Ratio	0.80 – 1.20
IgG anticardiolipin antibodies	2.6	GPL U/mL	<15.0
IgM anticardiolipin antibodies	3.1	MPL U/mL	<15.0
IgG anti-β2glycoprotein I antibodies	1.8	SGU	<15.0
IgM anti-β2glycoprotein I antibodies	5.0	SMU	<15.0

a. Comment on the results in Table 1. (5%)

b. Do the results provide a definitive explanation for the cause of her thrombosis? Justify your answer. (5%)

The patient had another pregnancy three years later which was covered with low molecular weight heparin and was uneventful. She subsequently began oral contraception. At the age of 41 she developed diverticulitis and underwent a laparoscopic right hemicolectomy at a

different hospital. Two days after the surgery she became short of breath and a CT scan of her chest revealed a pulmonary embolism. Intravenous unfractionated heparin at standard dose was initiated for treatment.

Serial APTT testing was employed to monitor the unfractionated heparin and the results during the first two days are shown in Table 2. The therapeutic range as APTT ratios for this reagent had been locally calibrated against anti-Xa levels to 1.5 – 2.7.

Table 2: APTT monitoring of UFH

Time point (hours)	APTT ratio	Further information
0	0.99	Baseline
6	1.10	
12	1.13	
18	1.60	Post one unit FFP
24	1.30	
30	1.15	
36	1.10	UFH dose increased
42	1.12	UFH dose increased
48	1.12	UFH dose at 50,000 IU/24h

c. Comment on the results in Table 2. (5%)
d. Discuss causes of this phenomenon. (10%)

Not knowing that the patient had been previously tested for thrombophilia at another hospital, a thrombophilia screen was requested and the results are given below in Table 3.

Table 3: Coagulation screen and thrombophilia assays

Test/assay	Result	Units	Reference range
Prothrombin time	10.9	s	9.0 – 13.0
APTT (LA-insensitive)	1.12	ratio	0.80 – 1.20
Fibrinogen (Clauss)	2.22	g/L	2.0 – 4.0
Antithrombin activity (chromogenic; FXa-based)	41	iu/dL	80 - 115
Antithrombin antigen	99	iu/dL	79 - 120
Protein C activity (chromogenic)	111	iu/dL	78 - 135
Free protein S antigen	65	u/dL	Female 68 – 133 Male 80 - 137
APTT-based APCR assay (1+4 dilution in FV deficient plasma)	2.61	Ratio	2.50 – 3.50
dRVVT screening test	1.00	Ratio	0.82 – 1.17
LA-sensitive APTT screening test	1.14	Ratio	0.83 – 1.18
IgG anticardiolipin antibodies	3.0	GPL U/mL	<12.0
IgM anticardiolipin antibodies	2.5	MPL U/mL	<12.0
IgG anti-β2glycoprotein I antibodies	3.2	SGU	<20.0
IgM anti-β2glycoprotein I antibodies	7.1	SMU	<20.0

e. Comment on the results in Table 3. (5%)

f. Discuss possible reasons for any clinically significant discrepancies between the results from Tables 1 and 3 and suggest and justify a diagnosis based on all the available information. (20%)

g. Critically evaluate methods to distinguish between type 2 sub-types of this disorder. (20%)

h. In view of the supratherapeutic dose of unfractionated heparin, discuss the possibility of the fibrinogen and APCR assays results being unreliable. (10%)

An anti-Xa assay was performed to quantify the level of unfractionated heparin, which gave a result of 2.1 IU/mL (therapeutic range: 0.3 – 0.7 IU/mL). Residual plasma from that sample was sent to a reference laboratory to confirm the antithrombin and anti-Xa levels. The results are given below in Table 4.

Table 4: Confirmatory results from reference laboratory

Test/assay	Result	Units	Reference range
Antithrombin activity	43	iu/dL	85 - 125
Antithrombin antigen	100	iu/dL	80 - 130
Anti-Xa	0.1	IU/mL	0.5 – 1.0

i. Discuss possible reasons for the discrepant anti-Xa results. (10%)

j. What are the options for treating this patient's pulmonary embolism? (5%)

k. Could Antithrombin Glasgow (p.425Arg > His) or Antithrombin Denver (p.426Ser > Leu) be the cause of the discrepancy between the antithrombin activity results from the assays used in the laboratories providing results for Tables 1 and 3? Justify your answer. (5%)

Unseen Case Studies

2.

A 43-year-old mother presented her 14-day-old girl to the paediatrician with slight fever and an unwillingness to feed. The daughter has previously been diagnosed with Down Syndrome *in utero.* Upon examination, the patient demonstrated a slightly enlarged liver and spleen, jaundice and hypotonia. The patient appeared slightly dehydrated. Initial haematological investigations provided the results shown in Table 1.

Table 1: FBC results

Parameter	Result	Reference range
WBC	32	6.0 - 21.0 x 10^9/L
Neutrophils	3.4	1.8 - 5.4 x 10^9/L
RBC	3.65	3.7 - 6.5 x 10^{12}/L
MCV	124	100 – 135 fL
MCH	***	28 – 40 pg
Haemoglobin	106	149 - 237 g/L
Platelets	63	150 - 450 x 10^9/L

a. Calculate the patient's Mean Corpuscular Haemoglobin (MCH) and, based upon her red cell indices, anticipate the patient's red cell morphology you would expect to see in the blood film monolayer. (5%)

b. Discuss the remaining full blood count results. (5%)

c. Evaluate the appearance of the accompanying Romanowsky stained blood film field shown in Figure 1. (20%)

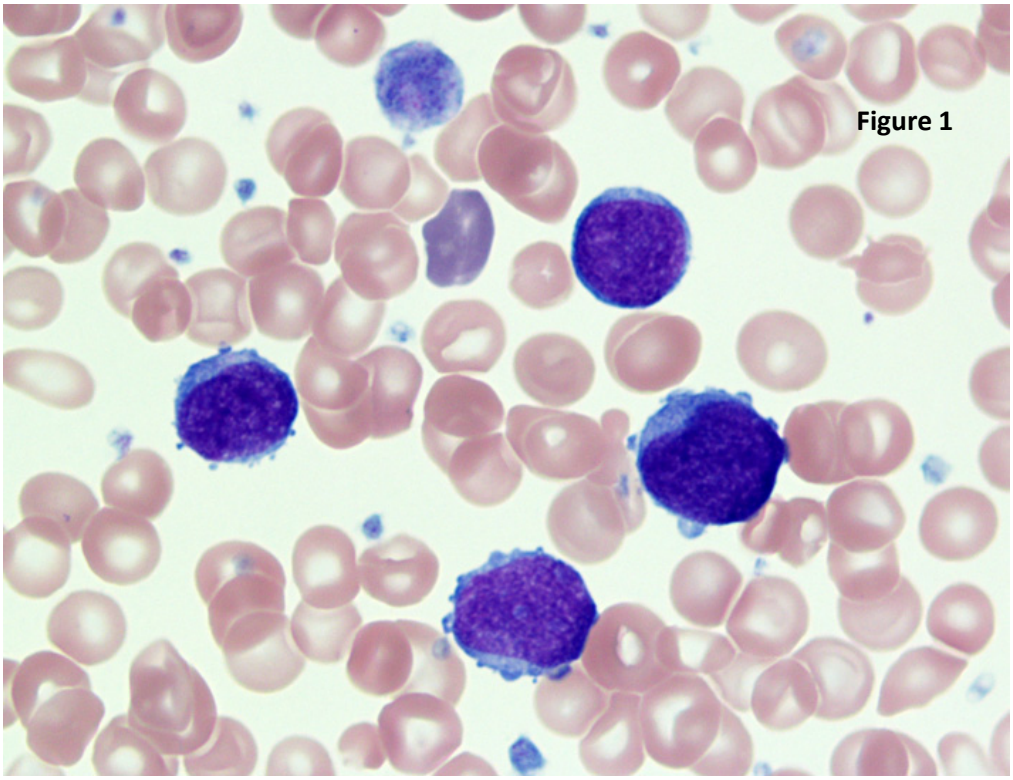

Figure 1

d. The peripheral blood blasts were 15%. Explain the significance of this patient's blast percentage. (10%)

e. Identify any additional molecular analysis that would contribute to the diagnosis. (20%)

f. Using the immunophenotyping data provided in Table 2, identify the cell population and propose a diagnosis for this patient. (20%)

Table 2: Blast cell immunophenotyping

Blast Immunophenotype	
Positive	CD34, CD56, CD117, CD13, CD33, CD7, CD4 (dim), CD41, CD42, CD36, CD61, CD71, TPO-R.
Negative	MPO, CD14, CD15, GPA

g. What are the potential haematological sequelae for this patient? (20%)

3.

A patient attends A&E with fever, shortness of breath and slight jaundice. They also reported haemoglobinuria. The laboratory results in Table 1 below were obtained:

Table 1: Haematology results

Parameter	**Result**	**Reference range**
Haemoglobin	102	133 – 167 g/L
RCC	3.5	4.3 – 5.7 x 10^{12}/L
Mean cell volume	97	80 – 98 fL
Hct	0.32	0.4 – 0.5 L/L
WBC	12.8	3.5 – 10.0 x 10^{9}/L
Platelets	200	140 – 400 x 10^{9}/L
ESR	5	<10 mm/first hour
Prothrombin time	12	10 – 12 s
APTT	35	25 – 35 s
Fibrinogen	1.9	1.9 – 4.0 g/L

a. Calculate the MCHC. (5%)

b. Comment on the results in Table 1. (5%)

A blood film was prepared and the data in Table 2 below were reported:

Table 2: Differential and film report

Differential white cell count		**Reference range**
Neutrophils	10.3	2.0 – 7.0 x 10^9/L
Lymphocytes	2.0	1.0 – 3.0 x 10^9/L
Monocytes	0.5	0.2 – 1.0 x 10^9/L
Eosinophils	0.1	0.02 – 0.5 x 10^9/L
Basophils	0.1	0.02 – 0.1 x 10^9/L
Red cell morphology comments:	Marked poikilocytosis, fragmented cells, blister cells	

c. Comment on these findings. What conclusions can you draw and why? (10%)

d. List and justify the next investigations you would consider in light of these results and the reported clinical details. (15%)

e. An osmotic fragility test was carried out using hypotonic saline and incubation for 30 mins. The curve obtained with this patient's blood was normal. Comment on this finding. Could this test be made more sensitive or informative? If so, how? (10%)

f. A G6PD fluorescent spot test was performed and demonstrated faint fluorescence. What conclusions can you draw and why? (10%)

g. At this point the result of a reticulocyte screen became available. The percentage reticulocyte count was 5%.

   (i) Using data already given, calculate the absolute reticulocyte count. (5%)

   (ii) Does this finding add to any of the existing information you have? If so, how? (5%)

h. After incubation with sodium nitrite and subsequently nile blue sulphate solution, followed by addition of MTT tetrazolium, cells were examined microscopically and approximately 4.5% of red cells contained blue-stained formazan granules. Comment on these findings. (10%)

i. How would a finding of 45% stained cells affect your interpretation? (5%)

j. A G6PD activity assay was performed at the time of the G6PD screen and a result of 5 eu/g Haemoglobin was recorded (ref range 7-10 eu/g Hb). The test was repeated with a fresh blood sample three weeks later and the result was 2 eu/g Hb. What diagnosis can you make for this patient, and what could have caused the two results to be discrepant? (10%)

k. What other tests or information may be useful in this investigation and why? (10%)