Inadequacy of oleic acid in erythrocytes as a marker of malignancies

Wood *et al* have reported that the stearate to oleate ratio in the lipids of erythrocyte membranes exceeds 1.0 both in healthy controls and in patients without cancer and is less than 1.0 in all patients with malignancies.¹ Similarly, the ratio is 1.0 or higher in patients without recurrent tumours and less than 1.0 in patients with recurrence. They suggested that this phenomenon might be caused by factors secreted from malignant tumours that increase the desaturation of stearate in fast growing cell systems.

Patients, methods, and results

The saturation index of erythrocyte membrane lipids (stearate to oleate ratio) was determined in seven healthy hospital employees (reference group), 13 patients with various non-malignant diseases (control group: symptomatic gall stones (six patients), acute pancreatitis (one), duodenal ulcer (one), urogenital disorders (three), and pneumonia (two)), and 20 patients with various solid malignant tumours (malignancy group: gastrointestinal cancer (seven), pancreatic cancer (four), urogenital cancers (four), and miscellaneous cancers (four)). The mean (SD) age in the reference group was 42 (9), in the control group 63 (18), and in the malignancy group 67 (12).

The erythrocyte membranes were isolated after freezing at -20°C , according to the method of Wood *et al*.¹ The membrane lipids were extracted with a mixture of two parts methanol to one part chloroform² and saponified in methanolic sodium hydroxide.³ The resulting free fatty acids were methylated with boron fluoride-methanol (Supelco reagent kit) and separated by gas-liquid chromatography on an all glass column with 10% SP-2330 on 100/120 mesh Chromosorb W AWA-185. Nitrogen (25 ml/min) was the carrier gas. The starting temperature was 180°C , programmed to increase at $4^{\circ}\text{C}/\text{min}$ to a final temperature of 220°C . The methylated fatty acids were also separated on an all glass column packed with 3% SP-2310 and 2% SP-2300 on 100/120 mesh Chromosorb W.¹

The oleic and stearic acids were separated almost completely by gas-liquid chromatography, whereas the method described by Wood *et al*¹ gave incomplete separation but higher ratios of stearate to oleate. The mean (SD) ratio in the reference group (0.91 (0.041)) was significantly higher ($p < 0.01$, Student's *t* test) than in both the control group (0.68 (0.14)) and the malignancy group (0.63 (0.14)), but there was no significant difference between the control group and the malignancy group. The groups differed, however, in age distribution, as the reference group comprised volunteers aged under 60 whereas the age in both groups of patients ranged from 35 to 90. The figure shows a scatter plot of stearate to oleate ratio versus age in the reference and control groups. The correlation coefficient between the ratio and age was -0.62 ($p < 0.01$), indicating that age is a significant factor in determining the stearate to oleate ratio.

Stearate to oleate ratio in total lipids from erythrocyte membranes plotted against age in seven healthy volunteers (●) and 13 patients with non-malignant diseases (○). $y = 1.0683 - 0.0055x$. $R = 0.62$.

Comment

Wood *et al* did not consider the effect of age in their analyses. This might have influenced their conclusion that the stearate to oleate ratio separated clearly patients with malignant tumours from other subjects. Furthermore, the separation of oleic and stearic acids by their method was clearly inferior to that obtained by our technique, a factor that might also have influenced the results.

We conclude that if the effect of age on the stearate to oleate ratio is taken into account such a ratio does not separate patients with malignant tumours from other subjects.

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Three further cases of Lyme disease

The first British case of Lyme disease with neurological complications was reported recently.¹ We report three further cases to emphasise that this is not an isolated finding and is therefore an important diagnostic consideration. These cases presented within a short time of the original patient, which suggests that the neurological complications of Lyme disease are more common in Britain than is realised.

Case reports

Case 1—Two days before admission a 23 year old man developed generalised aches and pains, neck stiffness, headache, and weakness of the left side of his face. A month earlier he had noticed a spreading erythematous rash on his abdomen, which had resolved spontaneously. There was no history of a tick bite. Examination showed a healthy man, who was afebrile, without neck stiffness, with a fading rash on his abdomen compatible with erythema chronicum migrans. Neurological examination showed bilateral lower motor neurone facial palsies. Cerebrospinal fluid examination showed 28 lymphocytes, a protein concentration of 3.2 g/l, and a serum *Borrelia burgdorferi* titre of 1/256. The patient made a complete recovery after treatment with parenteral penicillin and oral tetracycline.

Case 2—A 42 year old man first noticed pain in the mid-dorsal region, which radiated round the right lateral chest wall in a band like fashion; anteriorly at this level he noticed a small patch of numbness. A month later he developed malaise, weight loss, and left sided facial weakness. Two days later the right side of his face became weak and he complained of unpleasant tingling down the ulnar three fingers of both hands. There was no history of a rash but he remembered an insect bite some months previously. Examination showed bilateral lower motor neurone facial weakness in an unwell man without other focal neurological or systemic signs. Cerebrospinal fluid examination showed 498 lymphocytes, a protein concentration of 1.48 g/l, and a serum *B burgdorferi* titre of 1/1024. An oral course of phenoxymethylpenicillin 500 mg four times daily for two weeks improved his systemic symptoms and his facial weakness gradually resolved.

Case 3—A 10 year old schoolgirl was bitten by a tick but did not develop a rash. A month later she developed painful swollen retroauricular and submandibular lymph nodes followed by general malaise and daily occipital headaches. She subsequently developed a left lower motor neurone facial palsy. The cerebrospinal fluid was not examined. The serum *B burgdorferi* titre was greater than 1/256. Treatment with ampicillin and prednisolone resolved the systemic symptoms, and her facial palsy gradually improved.

Comment

All three patients lived or worked in the New Forest area of Hampshire. Only one patient (case 3) reported a definite tick bite and only one patient (case 1) developed erythema chronicum migrans, the unique skin lesion of Lyme disease. All three patients developed typical neurological complications of Lyme disease with lower motor neurone facial palsy and two had a cerebrospinal fluid lymphocytic pleocytosis.^{2,3} The systemic manifestations in all three patients responded dramatically to antibiotic treatment but we could not determine whether this influenced the recovery of the facial palsies.⁴

Ixodes ticks, the vectors of the spirochaete *B burgdorferi*, which causes Lyme disease,⁵ are common in England, and lack of awareness of the diagnosis may be responsible for the previous paucity of cases. The absence of a history of a tick bite or erythema chronicum migrans does not exclude the diagnosis as not all patients report these events. Lyme disease should therefore be considered in unexplained cases of cranial polyneuropathy, radiculopathy, and chronic lymphocytic meningitis. The diagnosis can be confirmed by indirect immunofluorescence on serum or by testing the cerebrospinal fluid for *B burgdorferi*.

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Effect of carbonic anhydrase inhibitors on glomerular filtration rate in diabetic nephropathy

Proliferative retinopathy and secondary glaucoma are much more common in insulin dependent diabetics with diabetic nephropathy than in those without. Carbonic anhydrase inhibitors are widely used to treat glaucoma because they reduce the rate of formation of aqueous humour. Recently we observed large reductions in glomerular filtration rate during treatment with carbonic anhydrase inhibitors in insulin dependent diabetics with nephropathy and glaucoma. We wish to call attention to this side effect, which seems to have gone unnoticed in the past, perhaps because of its reversible nature.¹

Case reports

CASE 1

A 31 year old man with insulin dependent diabetes complicated with nephropathy, arterial hypertension, proliferative retinopathy, and glaucoma secondary to iridocyclitis was admitted to hospital because of malaise, vomiting, and poor metabolic control. Ten days before admission he had started treatment with a carbonic anhydrase inhibitor, dichlorophenamide, 50 mg three times daily.

On admission he had a blood glucose concentration of 20.4 mmol/l, bicarbonate concentration of 15 mmol/l, no ketonuria, and a serum creatinine concentration of 357 µmol/l. His metabolic control and dehydration were corrected, but the serum creatinine concentration dropped to only 314 µmol/l and the glomerular filtration rate was severely reduced (10 ml/min. 1.73 m² eight days after admission) (table). Two months before admission his serum creatinine concentration had been 223 µmol/l and the glomerular filtration rate measured with a technique using edetic acid labelled with chromium-51 was 23 ml/min. 1.73 m².² As he did not have a urinary tract infection and the antihypertensive treatment (prazosin 7.5 mg/day, metoprolol 150 mg/day, and frusemide 250 mg/day) had remained unchanged we suspected that the carbonic anhydrase inhibitor had induced the acute deterioration in kidney function. This

treatment was stopped, and his clinical condition improved: five days later serum creatinine concentration had fallen to 230 µmol/l and three months later glomerular filtration rate was again 23 ml/min. 1.73 m².

CASE 2

A 41 year old man with insulin dependent diabetes suffering from nephropathy, arterial hypertension, proliferative retinopathy, and haemorrhagic glaucoma was admitted to hospital because of tiredness and poor metabolic control; he had a blood glucose concentration of 17.1 mmol/l and no ketonuria. Two months before admission he had undergone vitrectomy, but glaucoma had developed and acetazolamide 250 mg twice a day had been started. The table shows his kidney function before, during, and after acetazolamide treatment.

CASE 3

A 41 year old woman with insulin dependent diabetes suffering from nephropathy, arterial hypertension, and proliferative retinopathy visited the outpatient clinic for a routine check up. One week before she had developed iridocyclitis with secondary glaucoma and treatment with acetazolamide 250 mg twice a day had been started. We observed a slight rise in the serum creatinine concentration, and so the glomerular filtration rate was determined using ⁵¹Cr-edetic acid.² Three weeks later the intraocular pressure was normal, acetazolamide treatment stopped, and kidney function improved (table).

Comment

Intravenous injection of acetazolamide induces a slight (10-15%) reduction in the glomerular filtration rate in normal men.³ Micropuncture studies in rats have shown reduced proximal tubular absorption of fluid and electrolytes, suggesting that activation of the tubuloglomerular feedback mechanism is the main cause of the reduced glomerular filtration rate during administration of acetazolamide.⁴

Our cases show that carbonic anhydrase inhibitors may considerably reduce the glomerular filtration rate in diabetic nephropathy. The reduction in kidney function brought one patient close to the limit for starting dialysis treatment. The reversible decline in kidney function was not due to urinary tract infection, diabetic ketoacidosis, or arterial hypotension. All three patients received antihypertensive treatment, which remained unchanged during the observation period. This side effect of carbonic anhydrase inhibition may have escaped attention in the past because of its reversible nature or because serum creatinine concentration, which is a rather insensitive index, has been used to measure glomerular filtration rate, or both.⁵

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Kidney function, arterial blood pressure, and blood glucose concentration before, during, and after treatment with carbonic anhydrase inhibitors in insulin dependent diabetics with nephropathy and secondary glaucoma

	Case 1			Case 2			Case 3		
	Before*	During	After*	Before*	During	After*	Before*	During	After*
Glomerular filtration rate (ml/min. 1.73 m ²)	23	10	23	93	56	79	48	31	51
Serum creatinine (µmol/l)	223	314	230	146/96	119	95	135	151	138
Blood pressure (mm Hg)	147/93	155/90	150/90	19.5	135/85	150/86	122/90	107/86	111/75
Blood glucose (mmol/l)	8.1	11.8	11.6	7.0	10.5	3.7	3.2	8.4	

*Measurements made within four months before and four months after treatment with a carbonic anhydrase inhibitor.