Papers and Originals

Ten-year Haematological Follow-up: Mortality and Haematological Changes

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Summary: In a 10-year follow-up study of 543 men and 180 women seen in a community survey in 1958 mortality was examined in relation to the 1958 haematological data, and 91% of the survivors were re-examined. Analyses based on the 1958 haemoglobin and packed cell volume estimations suggested that those with values near the mean may subsequently have lower death rates. Higher death rates occurred in those having low serum iron levels. Comparisons of haemoglobin concentrations and of packed cell volumes in 1958 and 1968 show correlation coefficients (r) between 0.30 and 0.60 in various subgroups.

Introduction

The distribution of haemoglobin levels and packed cell volumes (P.C.V.s) in the general population have been studied in several surveys—for example, Kilpatrick and Hardisty (1961), Kilpatrick (1961), Elwood (1964), Elwood *et al.* (1967), and Campbell *et al.* (1968). Generally those subjects with low values receive investigation and treatment, and in most cases iron therapy will restore the haemoglobin level to "normal" values. Little is known about the relevance of the initial haemoglobin and P.C.V. levels to long-term prognosis; nor is it known how constant these haematological indices are in individuals over a period of many years.

This paper reports the haematological results of a 10-year follow-up of the Rhondda Fach sample whose 1958 data were reported by Kilpatrick and Hardisty (1961).

Procedure and Methods

The random sample of men seen in 1958 was stratified by occupation (miners, including ex-miners; and non-miners) and age (35-44, 45-54, and 55-64 years in 1958). The women were a random sample of those aged 55-64 years in 1958. In 1958 haematological results were obtained for 543 men and 180 women, this being 92.8% of the available sample; these are the subjects that were followed up between September and November 1968. For those who had died, information was obtained about the date and place of death, and in all cases this was confirmed by an examination of the death certificate. Subjects were seen either at a central clinic or in their homes

in the Rhondda, and an attempt was also made to see those survivors who lived elsewhere in England and Wales. All subjects were also examined for cardiovascular disease, respiratory disease, and arthritis, but only the haematological data are presented here.

A sample of venous blood was taken and 2.5 ml. placed in a bottle containing Sequestrene (sodium edetate) and gently mixed. The haemoglobin and P.C.V. estimations were usually determined on the following day, after storing the specimen overnight at 4° C. Haemoglobin was estimated as grammes per 100 ml. by the cyanmethaemoglobin method with an E.E.L. flow-through Spectra colorimeter. The instrument was standardized by a standard solution of cyanmethaemoglobin controlled by the British Committee for Standards in Haematology. P.C.V.s were measured by the microhaematocrit method (Hawksley).

Subjects with low results were offered investigation and treatment. Serum iron estimations were not done in 1968 though they had been estimated in 1958.

Results

Details of the 1958 population, the number that had died, and the number followed up in 1968 are given in Table I. In four cases the number of results is less than the numbers shown as seen in 1968 in Table I; complete haematological data were not available from three men and one woman. The mortality from all causes was examined in subgroups defined by haemoglobin level (Table II), P.C.V. (Table III), and serum iron concentration (Table IV) at the original survey in 1958. The overall mortality was higher in the miners (22%) than in the non-miners (17%). Higher mortality rates occurred with low haemoglobin levels in both occupational groups in men and also in the women. There was little evidence of any increased death rate with low P.C.V.s in men, and the excess mortality at these levels in women was only a very few deaths. There was a higher death rate with high P.C.V.s, and this trend is apparent in miners, non-miners, and women. In both sexes there was a considerably higher mortality rate with low serum iron concentrations (Table IV). Tables II-IV included deaths at all ages, though most occurred in the oldest age group (55-64 years in 1958). The mean haemoglobin levels, however, differed significantly in the six age/occupation groups in men (Kilpatrick and Hardisty, 1961). To standardize for this, and also as a means of grouping the data by unbiased and more meaningful divisions, we examined the mortality rates in relation to the number of standard deviations (S.D.) from the mean (of each sex/age/occupation group). The number of deaths in subgroups more than 1 S.D. from the mean is small. The

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results in Table V, however, suggest that the lowest mortality experience usually occurred in subjects with haemoglobin levels and P.C.V.s near the mean. The number of deaths in the 10 years was not sufficient to show meaningful differences between groups when analysed according to cause of death.

TABLE I.—Details of Population and Response Rates

Occupation Group	Age (in 1958)	No. Examined 1958	Died 1958-68	No. of Survivors 1968	No. Examined 1968	Response Rate (%)
Miners	35-44	90	6	84	81	96
	45-54	95	22	73	65	89
	55-64	93	34	59	57	97
Non-miners	35-44	85	3	82	80	98
	45-54	89	14	75	68	91
	55-64	91	28	63	57	90
Women	55-64	180	40	140	117	84
Total		723	147	576	525	91

TABLE II.—Deaths During 1958-68 and Haemoglobin Levels (g./100 ml.) in 1958

Haemoglobin (1958)	Miners		Non-miners		Women	
	No.	Dead (%)	No.	Dead (%)	No.	Dead (%)
< 10	0	—	0	—	7	4 (57)
10-12	4	2 (50)	5	1 (20)	18	4 (22)
12-14	60	18 (30)	45	11 (24)	102	22 (22)
14-16	179	35 (20)	169	27 (16)	50	8 (16)
> 16	35	7 (20)	46	6 (13)	3	2 (67)
Total	278	62 (22)	265	45 (17)	180	40 (22)

TABLE III.—Deaths During 1958-68 and P.C.V. in 1958

P.C.V.	Miners		Non-miners		Women	
	No.	Dead (%)	No.	Dead (%)	No.	Dead (%)
< 32	0	0	0	0	4	2 (50)
32-36	1	0 (30)	2	1 (10)	9	3 (33)
36-40	9	3	8	0	23	4 (17)
40-44	50	7 (14)	52	9 (17)	91	14 (15)
44-48	143	28 (20)	131	19 (15)	44	14 (32)
> 48	75	24 (32)	72	16 (22)	9	3 (33)
Total	278	62 (22)	265	45 (17)	180	40 (22)

TABLE IV.—Deaths During 1958-68 and Serum Iron (μ g./100 ml.) in 1958

		Serum Iron in 1958						Total
		0-50	50-100	100-200	200-300	300-400	Not Done	
Men	No.	18	177	224	81	37	6	543
	Deaths	6	50	38	8	5	1	108
Women	No.	17	72	62	17	9	3	180
	Deaths	7	17	13	2	0	1	40
		41	24	21	12	0	33	22

TABLE V.—Deaths During 1958-68 and Haemoglobin and P.C.V. Levels (in Standard Deviation (S.D.) Units) in 1958

		Below 2 S.D. from Mean	1-2 S.D. from Mean	Within 1 S.D. of Mean	1-2 S.D. above Mean	Above 2 S.D. from Mean
Haemoglobin:						
Women. No. of deaths (%)	..	4 (57)	4 (27)	27 (19)	5 (29)	0* (—)
Men. No. of deaths (%)	..	5 (28)	13 (22)	78 (19)	8 (15)	3 (33)
P.C.V.:						
Women. No. of deaths (%)	..	2 (50)	3 (30)	30 (20)	5 (28)	0† (—)
Men. No. of deaths (%)	..	4 (31)	8 (13)	75 (18)	15 (35)	5 (45)

* None "at risk." † One "at risk."

TABLE VI.—Haemoglobin Results for 406 Men and 116 Women Seen in Both 1958 and 1968

Age (in 1958)	Group	No.	Haemoglobin (g./100 ml.)			P.C.V. (%)		
			1968 Mean (S.D.)	1958 Mean (S.D.)	Correlation Coefficient r	1968 Mean (S.D.)	1958 Mean (S.D.)	Correlation Coefficient r
35-44	Miners	80	15.8 (1.26)	14.9 (1.07)	0.55	46.0 (3.49)	45.7 (3.08)	0.50
	Non-miners	79	15.7 (0.96)	15.1 (1.00)	0.32	46.0 (3.15)	45.5 (3.14)	0.30
45-54	Miners	65*	15.8 (1.33)	15.0 (1.12)	0.60	46.3 (3.84)	44.6 (3.36)	0.43
	Non-miners	68	15.4 (1.15)	14.8 (1.19)	0.37	45.1 (3.51)	45.4 (3.51)	0.48
55-64	Miners	57	15.3 (1.78)	14.6 (1.06)	0.44	45.6 (4.37)	45.2 (3.01)	0.34
	Non-miners	57	15.5 (1.38)	14.9 (1.20)	0.37	46.3 (3.41)	46.4 (3.27)	0.37
55-64	Women	116	13.8 (1.38)	13.4 (1.31)	0.57	41.2 (3.65)	42.2 (3.39)	0.52

* 64 for haemoglobin.

Haematological results are available for both 1958 and 1968 in 406 men and 116 women (Tables VI-VIII). These data show a slight increase in the mean haemoglobin concentration in each of the seven subgroups, but there is no consistent trend in the P.C.V. means. The correlation coefficient r for those with results in 1958 and 1968 varied between 0.32 and 0.60 for haemoglobin and 0.30 and 0.52 for P.C.V. In 1968 9 men (2.2%) had haemoglobin levels less than 12.5 g./100 ml. and 8 women (6.9%) had levels of less than 12 g./100 ml., these arbitrary levels having been used in the 1958 survey to designate the prevalence of anaemia.

TABLE VII.—Haemoglobin Levels (g./100 ml.) in 116 Women (Aged 55-64 Years in 1958) Seen in Both 1958 and 1968

Discussion

Previous haematological studies on the same individuals extending over a period of time have been based on a few volunteers (Pucher *et al.*, 1934), on patients in general practice (Fry, 1961), or on hospital patients (Beveridge *et al.*, 1965). The epidemiological study reported here gives the mortality experience of subgroups based on the haematological data at the first survey and also enables the consistency of the haematological measurements to be assessed after a 10-year period in the survivors.

The data suggest that, with both haemoglobin and P.C.V., those with values near the mean have a lower mortality experience. Those with values more than 2 S.D. from the mean experienced considerably higher mortality rates, but these rates are based on small numbers. Abnormal haemoglobin levels and P.C.V.s are usually defined as those below certain arbitrarily chosen values, and less attention is paid to the top end of the distribution. In our data most of the excess deaths occurred in men with high P.C.V.s. In men with a P.C.V. more than 1 S.D. above the mean there were 20 deaths against an expected number of only 10.6.

During the 1958 survey an attempt was made to find the causes of anaemia in all subjects with low haemoglobin levels by investigations in hospital or, if this was refused, by questioning subjects in their homes. As a result of this four men, but no women, were found to be suffering from diseases likely to be fatal, but in fact only two of these died during the

10-year follow-up. There is therefore little indication that the higher death rates with low haemoglobin levels were due solely to the inclusion of patients thought to be dying at the time of the first survey.

The distributions of serum iron concentrations in 1958 were the first obtained from random samples of the general population. The positive correlation between serum iron and mean corpuscular haemoglobin concentration was only +0.19 (Kilpatrick and Hardisty, 1961) and serum iron by itself was therefore not considered to be a satisfactory test for iron deficiency. There was little or no correlation between serum iron and age in the groups studied in 1958, though there is a considerable diurnal variation. The increase in the death rate found in both men and women with low serum iron concentrations (Table IV) is one of the most striking of our findings.

We are hesitant to present the statistical probability of these various mortality rates being different, and this hesitancy does not depend on the number of deaths with high and low values in 1958. It is due to the fact that we started with no particular hypotheses about these possibilities. In such circumstances large numbers of χ^2 tests do not carry the statistical probability that each implies. In fact, only a few such tests were significant at conventional levels. We have therefore not given probability values in the Tables, as these would have arbitrarily divided the results into significant and non-significant findings. Such statistical confirmation may bear little relation to the clinical importance of the findings. There is considerable difficulty in finding evidence during haematological surveys that anaemia, especially in women, is important in terms of symptoms (Elwood *et al.*, 1969), cardiorespiratory function (Cotes *et al.*, 1969), or as evidence of serious underlying disease (Kilpatrick and Hardisty, 1961; Elwood *et al.*, 1967). In view of this difficulty in showing that anaemia in the community matters our data are presented here as being of considerable importance if confirmed in other studies.

Even if the association of higher mortality rates and greater distance from the mean is confirmed it does not follow that these abnormal haemoglobin, P.C.V., or serum iron levels are the main aetiological factor in the higher mortality. In particular it would not be known whether raising the lower levels—

for example, by iron therapy—and reducing the higher levels (perhaps by phlebotomy) would reduce the mortality experience of these groups. Several surveys of haematological variates have been completed in recent years and follow-up studies on other populations can soon be done.

Comparisons of the 1958 and 1968 haemoglobin levels for the individuals seen in both surveys show that in 1968 the mean levels were slightly higher and that this difference was present in all subgroups (Table VI). There was no consistent trend in the small changes in P.C.V.s. It is therefore possible that the slightly higher haemoglobin levels were due to a change in technique between the surveys or the use of a cyanmethaemoglobin method in 1968. In 1958 haemoglobin was determined as oxyhaemoglobin, thus not measuring any methaemoglobin or carboxyhaemoglobin. When the results of individuals are compared (Tables VII and VIII) there is seen to be some correlation for both haemoglobin and P.C.V. in all groups. This correlation coefficient r varied between 0.30 and 0.60 (Table VI), which accounts for between 9 and 36% of the variation of the 1968 results. This is despite all those with low haemoglobin levels in 1958 being offered investigation and treatment for six months.

In the 1958 survey Kilpatrick and Hardisty (1961) designated anaemia to be less than 12.5 g./100 ml. in men and less than 12 g./100 ml. in women. With these criteria the prevalence of anaemia in those individuals seen in both 1958 and 1968 had fallen from 3.0% (12 cases) to 2.2% (9 cases) in men and from 11.2% (13 cases) to 6.9% (8 cases) in women. The overall prevalences of anaemia in 1958 in men (3.3%) and women (13.9%) were even higher (Kilpatrick and Hardisty, 1961), but, as one of the main findings reported in this paper is that those with low haemoglobin levels have a higher death rate, only those individuals who were seen on both surveys are used to compare the prevalence of anaemia. This lower prevalence could be due to a general change in the prevalence of anaemia or to effective treatment of those found anaemic in 1958. It could also be due to technical changes in measuring the haemoglobin level (mentioned above). This possibility is more likely, as changes in the proportion of subjects with a low P.C.V. in fact show the opposite trend. Again considering only those

TABLE VIII.—*P.C.V.s (%) in 116 Women (Aged 55-64 Years in 1958) Seen in Both 1958 and 1968*

		1958																									Total (1968)
		30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	
	30																										0
	31																										0
	32										1				1	1											3
	33	1															1										2
	34									1																	1
	35								1																		1
	36						1						1														3
	37										1																1
	38									1		1				2		1	2								9
	39									1	2	2	1	2	1					1	1						12
1	40									1	1	1	1	1	6		1										15
9	41											2	2	4	3	5								1			18
6	42									1		1	1	1	3	5			1								11
8	43											1			7	3	1										15
	44														1	1	1										8
	45														3	1											6
	46																										4
	47																										1
	48																										2
	49														1		1										2
	50																										1
	51																										0
	52																										0
	53																										0
	54																										1
Total (1958)		1	0	0	0	2	3	1	3	6	4	11	4	26	15	17	7	8	2	4	1	0	1	0	0	0	116

The correlation coefficient r is 0.52.

individuals seen in both 1958 and 1968 the numbers of women with P.C.V.s below 35% were 3 and 6 respectively; the numbers of men with P.C.V.s below 40% were 8 and 13 respectively. This increase in the number of subjects with low P.C.V.s in 1968 may be related to seasonal variation. Pucher *et al.* (1934), in four volunteers, found the highest P.C.V.s in late spring (the time of the 1958 survey) and some of the lowest levels in the autumn (the time of the follow-up in 1968). Clearly there are very considerable difficulties in determining any changes in prevalence in the distributions of haemoglobin or P.C.V.s.

Of 13 women below 12 g./100 ml. in 1958, three were again below 12 g./100 ml. in 1968 (Table VII). Of 12 men below 12.5 g./100 ml. in 1958, only one was again below that level in 1968. Using a similar definition, Fry (1961) in a follow-up of anaemic cases in general practice for 5 to 10 years found that one-third were still anaemic. Beveridge *et al.* (1965) studied 378 hospital inpatients with hypochromic anaemia; on follow-up over a third of the women and over a quarter of the men were still iron-deficient. There is thus considerable evidence that once anaemia has been detected these subjects are more likely to be anaemic subsequently. The figures from the present survey suggest that this likelihood is increased in the selected

subjects identified in general practice (Fry, 1961) and in hospital patients (Beveridge *et al.*, 1965) compared with the unselected populations studied in this report.

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Steady-state Plasma Levels of Nortriptyline in Twins: Influence of Genetic Factors and Drug Therapy

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Summary: Nineteen identical (monozygotic) and 20 fraternal (dizygotic) sets of twins between 45 and 51 years of age were given nortriptyline orally in doses of 0.2 mg./kg. body weight three times daily for eight days. The steady-state plasma concentrations of nortriptyline were calculated from the mean of the determinations for days 6, 7, and 8. Identical twins, not treated with other drugs, achieved similar steady-state plasma concentrations of nortriptyline in contrast to fraternal twins who were not given other drugs. The intrapair similarity in steady-state plasma concentrations was not found in identical twins simultaneously treated with various drugs during the experiment. Identical and fraternal twins treated with drugs containing barbiturates had considerably lower steady-state plasma concentrations of nortriptyline compared with untreated twins.

It is concluded that most of the variability in nortriptyline steady-state plasma concentration between persons who have not received drugs is genetically determined. Exposure to other drugs also influences the steady-state plasma concentration of nortriptyline, which in a given patient may therefore be determined by a resultant of genetic and environmental factors.

Introduction

It has recently been established that patients who are treated with the same dose of various tricyclic antidepressants such as desipramine (desmethylinipramine) or nortriptyline show great differences in their steady-state plasma levels (Hammer and Sjöqvist, 1967; Sjöqvist *et al.*, 1968). For desipramine (25 mg. t.i.d.) a range between 8 and 295 ng./ml. plasma has been observed (Hammer and Sjöqvist, 1967; Sjöqvist *et al.*, 1968) and for nortriptyline, given in the same dose, steady-state plasma levels have been shown to vary fortyfold between patients (this laboratory, unpublished). Extensive pharmacokinetic investigations led to the suggestion that the steady-state plasma level of these drugs is mainly determined by their rate of metabolism, since only small individual differences were found in the plasma protein binding, degree of absorption, and renal elimination of these compounds in man (Hammer and Sjöqvist, 1967; Sjöqvist *et al.*, 1968, 1969; Borgå *et al.*, 1969).

The metabolism of drugs in man may be influenced by both genetic (Evans, 1968) and environmental factors, such as exposure to other drugs (Levi *et al.*, 1968) and insecticides (Kolmodin *et al.*, 1969). In heavily drug-treated psychiatric patients it is impossible to evaluate the relative contribution of either factor to the quoted interindividual differences in steady-state plasma levels of tricyclic antidepressants.

This work was undertaken with a dual purpose: (a) to study the role of genetic factors in determining the steady-state plasma levels of nortriptyline, and (b) to assess the influence of concomitant drug therapy on this pharmacokinetic value.

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