Blood groups and disease: hard facts and delusions

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Abstract

The possibility that people who differ with respect to the A, B, and O blood groups might differ in their susceptibility to such diseases as carcinoma of the stomach, duodenal and gastric ulcer, pernicious anemia, and diabetes mellitus has been much studied of late. A critical analysis of the information so far collected shows, however, that much of it cannot be accepted without serious reservations and that some of it is probably worthless. Since blood groups are inherited, their distribution in small communities is sometimes determined by the predominance of certain family or ethnic units. It is extremely difficult to find a series of healthy people that will be truly comparable to a series of patients with a given disease. The statistical methods used can yield valid results only when sound methodology provides reliable data. Failure to observe this axiomatic principle explains much of the present confusion in research on blood groups and disease.

Reference


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BLOOD GROUPS AND DISEASE—HARD FACTS AND DELUSIONS

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Recent investigations on blood groups and disease, some with real significance, others without, may have proved confusing to many workers in medicine, genetics, and anthropology. Such investigations nearly always tended to establish or confirm the existence of a relationship between groups A, B, and O and certain diseases—carcinoma of the stomach, duodenal and gastric ulcer, diabetes mellitus, pernicious anemia, bronchopneumonia, toxemia of pregnancy, and others. The conclusions have been based, as a rule, on a comparison between a "pathological" series from a hospital, including from less than 100 to over 1,000 cases, and a "control" series of individuals—usually blood donors—coming from the same areas as the patients. Differences in blood-group frequencies between such pathological and control series agreed in certain cases, disagreed in others, and were in general of the order of from 3 to 8%.

On the strength of this evidence it has been concluded that "persons belonging to different blood groups may differ substantially in their susceptibility to certain diseases of adult life." This conclusion has apparently received wide acceptance. Medical journals, such as the British Medical Journal, have acknowledged it editorially and have commented approvingly that work of this type "encouraged many other workers to enter the field."

The possibility that people who differ with respect to the A, B, and O blood groups might differ in their susceptibility to such diseases as carcinoma of the stomach, duodenal and gastric ulcer, pernicious anemia, and diabetes mellitus has been much studied of late. A critical analysis of the information so far collected shows, however, that much of it cannot be accepted without serious reservations and that some of it is probably worthless. Since blood groups are inherited, their distribution in small communities is sometimes determined by the predominance of certain family or ethnic units. It is extremely difficult to find a series of healthy people that will be truly comparable to a series of patients with a given disease. The statistical methods used can yield valid results only when sound methodology provides reliable data. Failure to observe this axiomatic principle explains much of the present confusion in research on blood groups and disease.

A critical analysis of the evidence so far collected shows, however, that much of it cannot be accepted without serious reservations and that some of it is
probably worthless. It is the object of this paper to show why this is so and, it is hoped, thereby incline research workers engaged in the study of the problem to adopt a sounder methodology in their investigations.

The study of the distribution of genes A, B, and O in various groups, diseased or healthy, is fraught with difficulties of which many statisticians and clinicians seem unaware. This is explained by simple reasons whose importance, however, is constantly underestimated: when a difference is found between two samples comprising, say, 500 or 1,000 individuals from the same locality, there is no way of telling to what extent it should be attributed to (1) genuine differences in the ethnic and racial make-up of the two samples; (2) the hazard inherent in the composition of small samples, i.e., sampling errors; (3) errors of technique; and (4) other causes, such as, for example, a possible relationship between blood groups and disease. These four possible sources of error have deliberately been listed in this order for reasons which will immediately become apparent.

**Differences in the Ethnic and Racial Composition of Samples**

Everybody knows that populations at the four corners of one continent, or even of one country, will show differences of all kinds, presumably also of a biological nature. But it is only the anthropologist who seems to be constantly aware of the differences that exist even within populations in a relatively small area.

Dr. A. Kopec, who is, with Dr. A. E. Mourant, an authority on the distribution of ABO genes in Great Britain, has shown that the distribution of gene A varies in that country at least between 19.87 and 29.44% and that of gene O between 64.52 and 72.87%.

Such natural differences are even greater in other countries, for example, in Austria, where the frequency of gene O, as found by Hoche and Moritsch in 1,000 individuals from Vienna, was 33.1%, while that found by Nowak in 976 subjects varies in that country at least between 19.87 and 29.44%, and that of gene O between 64.52 and 72.87%.

Such natural differences are even greater in other countries, for example, in Austria, where the frequency of gene O, as found by Hoche and Moritsch in 1,000 individuals from Vienna, was 33.1%, while that found by Nowak in 976 subjects was 46.4%. The frequency of gene B was 20.1 and 11.8% respectively.

While the clinician may attribute differences of this order to disease, the anthropologist with personal experience in blood grouping will expect them to occur naturally even in small populations. Any doubt on this point may be quickly removed by a look at Boyd's, Mourant's, Steffan's, or Wiener's tables, which show variations of the order of 5 to 20%, in the distribution of the ABO genes within most countries and even most cities.

This is not surprising, since the part played in the composition of a sample by hazard, inbreeding, genetic drift, ethnic and racial differences, and other factors may result in differences of precisely this order. I have shown elsewhere with Vestemeanu to what extent the examination of populations of even small, isolated villages, presumed to be unmixed, can lead to false conclusions because of the presence within the sample of whole families belonging to the same blood group. Hospital populations are suspect by definition, and so are populations composed of blood donors.

A difference of 5 or even 10% between two series cannot be attributed to any single cause unless there is reasonable evidence that the samples are statistically comparable. Such evidence is always very difficult, and sometimes impossible, to obtain. Inferences drawn from differences in the distribution of blood groups must therefore remain of a much more limited validity than certain workers attribute to them.

**Statistical and Technical Pitfalls**

The distribution of blood groups has usually been studied without due regard to proper statistical methodology or, until recently, without the help of the statistician. It is therefore not surprising that many investigations fail to take into account the role played by errors from pure chance or bias in the composition of the samples.

As a contribution toward the use of suitable statistical methodology in blood-group investigations, I prepared, in 1945, under the joint auspices of the Institute of Anthropology in Geneva and the Central Institute of Statistics of Romania, a study of the margin of error to be expected in samples of different sizes. The tables prepared show that when one works, for example, in a population with an expected gene A frequency of 45%, and the sample examined includes 2,500 individuals, the sampling error may be as high as 3.89%. But if the sample includes less than 200 people, the sampling error may be as high as 15%.

The methodology for the calculation of the margin of error outlined in the above-mentioned study represents merely the application of well-known statistical principles to the study of blood groups. What even the biologist with an aversion to mathematics will conclude from it is that the margin of error in carefully controlled experiments involving relatively unmixed samples of 1,000 individuals may be from 2 to 4%, simply because of the play of pure chance in the selection of such samples. The uncontrollable source of error further reduces the value of the absolute reliance sometimes placed on blood-group investigations.

To the foregoing must be added yet one other source of error too often overlooked: the technical error. It is a common assumption that from a technical point of view blood tests are always reliable. This is not so. Salber reported in 1950 an error of 2.1% in a sample of 1,698 soldiers originally tested by the Swiss Army and later retested by the Swiss Red Cross.

In 1952, Robinette and Foreman, also showed that technical errors occur more often than is generally thought. But by far the most impressive report is that of Richmond and co-workers, who showed that the error in the original blood grouping of 2,050 men of the United States Armed Forces was not, as many have assumed, less than 2%.
Forces and Air Force was as high as 8.5%. Recently evidence was advanced by Edwards to show that as many as 10% of infants with blood group AB are sometimes wrongly classified as having group B.

The latest report from the United States on the subject shows that, in the state of Illinois, 45 out of 205 blood banks required to test a sample of blood failed to identify it correctly and considered that it belonged to group B when, in fact, it belonged to group AB.

Obviously, under strict laboratory conditions, grouping errors do not occur as often as in the field, and, in fact, Bowley and Dunsford showed that the error in their laboratory tests was of the order of 2 to 5 per 1,000, while Mourant claims the outstanding achievement of an error of only 1 per 1,000. However, the findings of Richmond and his collaborators and the report of Salber should serve as a warning. In the two cases, blood-grouping errors may easily have resulted in fatalities, yet the error was as high as from 2 to almost 9%.

It is, of course, unlikely that every sample is marred by an error of similar magnitude. The fact remains, however, that no sample is completely free from technical errors.

**Critical Analysis**

Three important conclusions emerge from the foregoing remarks. First, a clear distinction should be made between control series used in the study of blood groups and those used in other fields of biological and medical research. Ethnic and racial factors may play an outstanding role in the distribution of ABO genes within a sample and often account for differences of from 5 to 20% within the population of the same country or the same city. In other fields of medical research such ethnic differences may not exist.

Second, statistical sampling errors may often be responsible for differences of up to 4% between samples of over 1,000 individuals, when the samples are selected in accordance with statistical theory, and may be even higher when, through inappropriate sampling procedures, some bias is inadvertently introduced.

Third, technical errors of the order of 2 to 9% may occur in blood groupings, although modern methods of serologic investigation reduce the margin of error to less than 1%. Such results, however, can be obtained only in well-equipped laboratories, and the fact remains that many mass investigations are less accurate.

No conclusion should be accepted by a scientist unless proper regard can be paid to the effect of these sources of errors on the ultimate results. The biologist concerned with blood groups is, therefore, always working against great odds.

Let us, with these limitations in mind, examine the evidence in favor of the existence of a relationship between disease and the ABO blood groups.

Many attempts have been made over a period of years to find such a relationship, but without conclusive results. While it was Struthers who attracted for the first time, in 1951, the attention of the statistician by claiming a relationship between bronchial pneumonia and the frequency of blood group A, it was the study by Aird and co-workers, undertaken in 1953, that lead to the conclusion that "the frequency of blood group A is greater and the frequency of blood group O less in patients suffering from cancer of the stomach than in the general population of the locality in which they live." Aird and Bentall compared, with the statistical collaboration of Roberts, the ABO blood groups of 3,632 individuals suffering from carcinoma of the stomach with the blood groups of individuals in a control series composed of subjects free from the disease or suffering from other diseases. The pathological series was broken down into seven subseries: Newcastle, 101 cases; Leeds, 217; Manchester, 771; Liverpool, 207; Birmingham, 100; London, 1,406; and Scotland, 473 (the total of 3,632 comprises several other small series). In all the series composed of persons suffering from carcinoma of the stomach, blood group A was significantly higher than in the control series. Here are the percentages for the seven series in the order quoted above:

- 43.6 cancer, 37.4 control;
- 47.9 cancer, 40.3 control;
- 44.5 cancer, 38.4 control;
- 44.7 cancer, 39.6 control;
- 57.0 cancer, 44.4 control;
- 46.0 cancer, 42.2 control;
- 38.4 cancer, 32.5 control.

The consistency of these findings is impressive. However, as will be seen later on, a review of the literature of similar data has failed to show equally consistent results. The differences attributed to cancer being of a low order (4 to 8%), and taking into account the magnitude of variations that have to be expected in any case because of natural differences in the genetic make-up of populations and because of inevitable sampling errors, one cannot fail to be surprised at the consistency of the result, the more so that the differences between the various parts of the country are substantial: group A varies in the cancer series from 36.4% in Scotland to 57.0% in Birmingham and in the control series from 33.5% in Scotland to 44.4% in Birmingham. Could not the results of this investigation be attributed to differences in the genetic make-up of the groups studied, to sampling errors, or to some other unknown consistent bias?

The question gains pertinence in the light of the divergent conclusions arrived at by different clinicians after the original investigations of Aird and associates.

Speiser, who examined 1,146 individuals with gastric carcinoma and a control series of 10,000 other individuals in Vienna, failed to find any association between blood groups A, B, and O and the disease, nor did Wallace, who examined 299 individuals with gastric carcinoma against a control series of 7,418 individuals in Glasgow.

Walther and associates, in analyzing 1,000 consecutive cases of malignant disease collected in three years at the Whipps Cross Hospital, Leyton-
significant differences from the general population.

cinoma of the stomach in Sydney in 1956, reached
between gastric carcinoma and the ABO blood groups

the conclusion that "the initial suggestion of Aird
et al. (1953) that blood-group A predisposes to the
development of gastric carcinoma ... does not seem

to be valid, but it appears that the association be­
tween gastric carcinoma and the ABO blood groups
is related to site only, and that the blood group

is related to site only, and that the blood group
gene B appears to be without localizing signifi­
cance." And Billington concludes that "it is possible
that the results may be brought about by bias in
collecting the samples."

Billington had reported that the ABO blood
groups of patients with gastric carcinoma differed
according to whether the lesion was in the pre­
pyloric area, the body of the stomach, or the cardiac
area. In a letter to the Lancet in 1956, Wiener
commented, "To me this suggests that, in assigning
the cancer to one or other area, the investigator was
subconsciously influenced, in borderline cases, by
his prior knowledge of the patient's blood group,"
and asked, "Can Dr. Billington exclude this possi­
bility?" Billington's answer, 14 published in the same
issue, was, "I admit I am unable to exclude the
possibility that the figures in relation to the site of
gastric carcinoma might have been influenced in
borderline cases by knowledge of the patient's blood­
group."

Jennings and co-workers, 13 who examined 119
persons suffering from carcinoma of the stomach,
concluded, "There was no excess of group A in
carcinoma arising in the body of the stomach, and
hence this material provides no support for the
hypothesis that group A is associated with atrophy
of the acid-producing mucosa and achlorhydria."
However, the number of cases is so small that this
particular investigation can have but a very limited
value, the more so because an association between
group A and carcinoma of the antrum was found
only in men.

These results were contradicted by Haddock and
McConnell, 15 who found "a higher incidence of
group A when the growth arose in the body or
cardiac end of the stomach than when it arose in
the pylorus and the antrum, and that this applies
in both sexes. " Commenting on the paper by Jen­
nings and co-workers, the authors state, "We have
comparable data which do not support this finding
and which, in fact, would appear to contradict it."

In reply to this, Balme, 16 in a letter to the Lancet,
in 1956, suggested, "This difference between us
could be due to a statistical freak of chance; a more
probable explanation lies in the selection of cases
and the method of classification."

Further doubts as to the existence of a relation­
ship between group A and carcinoma of the stom­
ach arise from the fact that, while Stocks showed
in 1950 that mortality from the disease was greater
in the northern than in the southern towns of Eng­
land, the proportion of group A is, contrary to expecta­
tions, considerably higher in the southern than in the northern parts of the country.

These, then, are the grounds on which the associ­
bation between group A and carcinoma of the
stomach was claimed to have been definitely "demo­
strated." Whether some kind of an association
between blood group A and carcinoma of the
stomach actually exists remains, therefore, a matter
of pure speculation. There is no conclusive evidence
for or against it.

Duodenal and Gastric Ulcer

The case for an association between some forms
of peptic ulcer and groups A, B, and O may appear
to be more convincing. Aird and associates 12
claimed in 1954 that in 3,011 cases from three cen­
ters in England the frequency of blood group O
was significantly higher in patients with peptic ulcer
than in the control series. Since then, the presumed
relationship was confirmed by Westland and
Heistö 13 in 1955, Koster and co-workers 14 in 1955,
and, partially, by Peebles Brown and associates 15
in 1956, and Buckwalter and associates 16 in 1956.

However, when finer methods were applied to
the study of the relationship between blood groups
A, B, and O and duodenal and gastric ulcer, the
picture which emerged was less clear. Clarke and
co-workers, 17 in 1955, examined 1,237 carefully
selected patients from three hospitals in Liverpool
against 15,877 other patients apparently free from
ulcer in the same hospitals and found an association
between duodenal ulcer and group O but not be­
tween the same group and gastric ulcer. A year
later Buckwalter and others, 18 in the United States,
found an association between group O and both
duodenal and gastric ulcer. Clarke and co-workers
combined their data with those provided by Aird
and co-workers in 1954 and were able to confirm
only the existence of a relationship between group
O and duodenal ulcer.

These differences are somewhat disturbing; nei­
evertheless, the findings concerning peptic ulcer
are more significant than those concerning cancer
for two reasons: 1. In cancer the differences were,
in general, of the order expected by the anthropol­
ist in any routine investigation. The difference
was more significant in peptic ulcer, since in some series
it exceeded 17% and suggested that persons having
blood of group O are about 37% more prone to have
duodenal ulcer than persons of other groups. 2.
While the cancer studies resulted in a picture of
confusion, an association between duodenal ulcer
and group O has been found by several workers.
It is only the study of gastric ulcer that has led to
contradictions.

The evidence therefore tends to suggest that a
relationship between group O and duodenal ulcer
actually exists. It is uncertain whether one can be
more definite and consider that the existence of an
association has been proved beyond doubt. As with
cancer, the question of the control series must
remain a cause for serious misgiving. Aird and co-workers and Clarke and co-workers have painstakingly listed some of the limitations. To those already mentioned others must be added. For example, the methodology which enabled these authors to combine their cases, and which is said to permit other workers to do so, is not acceptable to the anthropologist, since it would tend to render the control series so highly heterogeneous that, in fact, they would represent a purely artificial, unreliable point of reference. Köster and associates, for example, speaking of cancer, admitted that their patients with gastric diseases may not be comparable "with the general population represented by B. Anderson's figures." Their paper lists 10 cases of carcinoma of the stomach, classified according to the ABO blood groups, with the explanation, "These small numbers are inadequate for analysis, but they are mentioned so that they may be added to those recorded by others." Such an approach will horrify an anthropologist. The idea that cases from various parts of the world can be put together may appeal to the clinician beset with the problem of finding enough pathological data to satisfy the statistician. But with what will such a series be then compared, since the distribution of blood groups has been shown to vary enormously not only from one part of a continent to another but also between different regions of the same country?

Whether doubts about the association between blood groups A, B, and O and peptic ulcer are justified cannot be determined by the evidence at present available. One satisfactory method of determining an association, however, was brought to the attention of the clinician by the geneticist, namely, to demonstrate associations in sibships. As Clarke and co-workers pointed out in 1956, while "controls can be unsatisfactory in that a population of mixed origin may contain elements with a high frequency both of group O and of duodenal ulcer without the two being causally connected . . . . sibship studies where the unaffected sibs act as controls are not subject to this criticism." Clarke and co-workers undertook in 1956 a study of 295 sibs of patients with duodenal ulcer. Their conclusion was, "An analysis of these gives no evidence to support the hypothesis that a group O individual is more likely to have a duodenal ulcer than are his A, B, or AB sibs." They conclude, "This result could be regarded as evidence in support of the suggestion that the previously found association was due to racial stratification within the populations concerned." This, however, is by no means certain, and it will be necessary to make other similar investigations before definite conclusions can be drawn.

Yet another potentially interesting line of approach is the study of the geographical distribution of peptic ulcer and of blood groups A, B, and O. If the claim that duodenal ulcer is somehow associated with blood group O is well founded, a high incidence of the disease in areas with a high proportion of group O is to be expected. Figures for Great Britain published by Stocks in 1948, however, do not confirm this expectation. While blood group O is known to be more frequent in Scotland and in northern England, peptic ulcer appears to be more frequent in the South than in the North. However, since the rates per 10,000 comprised other forms of dyspepsia, they must be treated with reservation. The finding is nevertheless challenging and opens up new and interesting possibilities in the study of a so far little-explored field.

Other Diseases

A number of attempts have been made to find a possible association between blood groups A, B, and O and other diseases. The general background for such attempts is nearly always the same as that of those reviewed above. The investigators evidence partial or total unawareness of the magnitude of ethnic differences in the blood-group composition of populations and, not infrequently, unsound methodology.

Pernicious anemia was studied in 1921 by Buchanan and Higley, who rightly concluded from their data that there was no association. Aird and co-workers in 1956, decided, "In retrospect the percentage frequencies . . . do suggest an excess of group A." These authors studied 490 cases from various parts of Great Britain and added, for the analysis, 111 cases from Copenhagen, as recorded by Köster and associates in 1955. Their conclusion was that the data show "with fairly high significance that pernicious anemia is commoner in persons of group A than in persons of group O." This claim cannot be accepted because of the discrepancies in results obtained in different hospitals (the samples were, in any case, too small). Furthermore, conclusions based on the combination of all the samples into one larger one have no value since there can be no acceptable control series with which to compare the findings. The picture is further confused by Macleod's reminder in 1954 that as early as 1937 he had found some evidence of a possible association between groups B and AB and pernicious anemia.

Diabetes mellitus was studied in 1956 by McConnell and others in 1,333 patients who came from two different areas of Liverpool (since it had been found that the data for the whole of Liverpool were not homogeneous), and also from Oxford, which contributed 500 cases. These are not ideal conditions for an investigation. The authors concluded that "the outstanding difference that has emerged is that between the sexes. There is fairly strong evidence that diabetic men show an excess of group A, whereas diabetic women show no significant difference from the control series." This, again, is disturbing. The authors are obviously aware of the risks involved in their analysis, since they comment, "We have an additional reason for caution. We have compared results with Dr. J. Craig and Dr. I. Wang, who have carried out a similar investigation on
diabetes mellitus at the Victoria Infirmary, Glasgow. They do not find an excess of group A in either male or female diabetics.

A splendid example of the dangers inherent in this type of research is provided by investigations on toxemia of pregnancy. Starting with the general, but unwarranted, assumption that a relationship between blood groups A, B, and O and several diseases had been definitely established, Pike and Dickins \(^ {12} \) set out to investigate a possible association with toxemia of pregnancy. In a series of 3,651 consecutive deliveries, 541 patients with toxemia were examined. The conclusion was that “there is a marked preponderance of group O in the toxemic patients.” Roberts, who advised on the statistical analysis of the data, was of the opinion that the difference was “highly significant.”

In 1955, the material was “re-scrutinized, with stricter criteria for the diagnosis of toxemia” by Dickins, \(^ {12} \) with the result that “the significance of the difference was reduced but [that] the evidence remained fairly strong.” However, a second series, which comprised deliveries from May, 1954, to December, 1955, did not confirm the original result and Dickins and associates \(^ {12} \) conceded in 1956 that “it must now be concluded that we have no acceptable evidence that toxæmic and non-toxæmic mothers differ in their ABO frequencies.” Similar results have been reported by Pearson and Pinker. \(^ {19} \) who failed to find a relationship between group A and toxemia in 675 cases from a series of 11,086 pregnancies.

What is of particular interest in this instance is not that an error has been made and corrected—a process that must always occur in medical research—but what lies behind the comment of the authors that “some mystery remains, however, a mystery we cannot explain, as to why the original series gave the result it did.” There is no definite answer to this question, but in all probability there is no mystery to be explained either. When one works with samples of about 500, differences of the order that may suggest that a special factor of influence is at play may be expected simply because of the interplay of such ever-present factors as differences in the ethnic and racial origin of the individuals who compose the samples, genetic-drift, inbreeding, technical errors, and a variety of others. Much time, effort, and money is spent in making sure that results obtained in research on the distribution of blood groups tend to apply accurately observations, reliable laboratory work, and common sense.”

Valid data can only be obtained if more regard is paid to sound methodology. First and foremost, extreme caution must be exercised in insure on this tendency is entirely justified: “While many papers suffer from the author’s lack of knowledge of statistical methods, more suffer from their misapplication. Knowledge is no substitute for understanding, and mathematics is a poor substitute for accurate observations, reliable laboratory work, and common sense.”

Until more trustworthy evidence is available, judgment should be reserved. The wide and unqualified support given to the view that an association exists between groups A, B, and O and certain diseases may be proved warranted by future research, but at present it is based largely on speculation.

Summary and Conclusions

A number of authors have claimed to show that an association exists between blood groups A, B, and O and certain diseases: carcinoma of the stomach, duodenal and gastric ulcer, pernicious anemia, diabetes mellitus, and toxemia of pregnancy. Proof of the existence of this association would open new horizons in pathology and shake the foundations of that part of anthropology which is based on the study of blood groups. It would not appear that this association—although vigorously claimed and apparently widely accepted by clinicians—has yet been established. Most investigations have led to contradictory results and are open to objection on methodological grounds.

There is a general lack of awareness of the large differences in the frequency of blood groups that occur between two or more samples from the same locality. Two samples of 500 to 1,000 individuals from the same area or country may show differences of 5 to 25%. These may be due to (1) natural variations in the frequency of blood groups within a limited area (5-20%); (2) sampling errors (5-20%); or, occasionally, (3) technical errors (2-5%). Nevertheless, differences of from 4 to 8% have been considered proof of the existence of an association between blood groups A, B, and O and certain diseases.

References


