Pattern of inbreeding in thalassemia affected families resident in Tuman Leghari district Dera ghazi khan

Ayaz Ahmad, Asma Saeed and Dr. Amin-Ud-Din

Abstract
The inbred marriages are highly acceptable in many populations of human beings but their prevalence and configuration vary depending on ethnicity, religious conviction, learning and socioeconomic circumstances of relevant population. Such social contracts are reported as the most important cause of enhancing the occurrence of the hereditary disorders by recessive autosomal, especially Thalassemia. The challenges of hereditary diseases trouble in the population calls for the progress of anticipation programs. But the implementations of different means require the information about types and prevalence of hereditary diseases and family system in population. This study focuses on inbred marriages and genetic diseases in the general population of Tuman Leghari resident in district D.G. Khan, Punjab, Pakistan. Ethnically the highest positive response was found in Saraiki (other than Baloch) 47.30% than Leghari (32.62%) or Khosa (20.44%). The Saraiki other than Baloch, Khosa and Leghari families were 47.30%, 32.62%, and 20.44% respectively.

In total samples, representing the lower, middle and higher levels of male education comprised Leghari 120 (29.56%), Khosa (131 114 (19.95%), Khosa 133 (34.72%) and Saraiki other than Baloch 146 (35.96%). The Saraiki other than Baloch, Khosa and Leghari families were 47.30%, 32.62%, and 20.44% respectively.

The calculated mean inbreeding coefficient (F) for the total sample was 0.0287. Three groups formed on male education in order to assess the effect of education on the inbred marriages.

In total samples, representing the lower, middle and higher levels of male education comprised Leghari 114 (19.95%), Khosa 133 (34.72%) and Saraiki other than Baloch 184 (45.32%) couples, respectively.

Female education found to be statistically significant in Khosa group.

The effect of socioeconomic status of male on marriages was also studied; the sample was distributed in three groups on the basis of socioeconomic status of male at the time of marriage. The total sample representing the lower, middle, and higher levels comprised Leghari 120 (29.56%), Khosa 131 (32.62%) and Saraiki other than Baloch 155 (38.18%). The statistical analysis uncovers significant results in total and Leghari ethnic groups. In order to find the impact of socioeconomic status of female on inbred marriage, the sample was divided into three groups based on female socioeconomic status at the time of marriage. The total sample representing the lower, middle, and higher levels comprised Leghari 108 (26.60%), Khosa 152 (37.44%) and Saraiki other than Baloch 146 (35.96%). The statistical analysis uncovers significant results in total sample, Leghari and Saraiki other than Baloch ethnic groups. During the survey, the number of thalassemia couples were 26 (66.67%) respectively. The affected couples with Thalassemia were found widespread in the sample. On ethnic base, 33.33%, 10.26% and 56.41% of total afflicted couples were found to be related with the Leghari, Khosa and Saraiki other than Baloch groups respectively. In the groups of afflicted couples based on genetic diseases, inbreeding was found 100% in thalassemia.

Data on children in Leghari, Khosa and Saraiki other than Baloch were statistically significant in both affected and non-affected. In case of data on number of children in Leghari, Khosa and Saraiki other than Baloch in albino were found non-significant and in thalassemia were highly significant in chi test. Awareness about thalassemia and behavior of parents, relatives, society toward Thalassemics were found statistically significant.

Keywords: Inbreeding, outbreeding, Tuman Leghari, Khosa, Saraiki other than Baloch, thalassemia

Introduction
Inbreeding
Inbreeding marriages are described as a social contract among the individuals which have blood relations. This includes such type of relations which are termed as first cousins,
second cousins and distantly related. In a few groups, the most elevated inbreeding are come to with relational unions between double first cousins which are practiced in Arabs and uncle-niece unions are likewise by and by among South Indians [1].

Inbreeding coefficient (F) is defined as the measure of the extent of alleles with indistinguishable duplicates which are communicated in the posterity of inbreeding from both of the parents. If the inbreeding coefficient (F) is equal or more than 0.0156 then it is considered as an inbred marriage. This incorporates such kind of unions termed as first cousins, second cousins and remotely related [2].

The commonness of inbreeding and rate of inbred marriages fluctuate broadly from population to population and groups, contingent upon ethnicity, religion, society at nearby level and topographical dissemination of the populations [3].

Inbreeding relational unions likewise regularly happens among the emigrants people from profoundly inbreeding nations and locales, similar to Lebanon, Turkey, Pakistan and North Africa, which are presently occupant in Australia, North America and Europe [4-5].

Hereditary disorders

Human hereditary disease is brought on by variations in the genes from the hereditary materials of the normal people. There are four kinds of hereditary abnormalities like single-gene disorder, chromosomal disorder, multifactorial and mitochondrial disorders. Single gene disorder is caused by a single mutant gene. Such disorder can be transferred in many generations by many ways. This abnormality is brought on basically by deletion or addition of the DNA sequence in single gene [6-7].

Single gene disorder

A single gene disorder is the consequence of a single transformed allele. Single gene disorder can be gone on to resulting generations in a few ways. Genomic engraving and uniparental disomy, notwithstanding, may influence legacy designs. Two duplicates of the gene must be transformed for a man to be influenced by an autosomal recessive disorder. Illustrations of this kind of disorders are Albinism, medium-chain acetyl-CoA dehydrogenase lack, and thalassemia disorder. Certain different phenotypes, for example, wet versus dry earwax, are likewise decided in an autosomal recessive manner [8].

Thalassemia

Thalassemia is an acquired disorder portrayed by irregular and low generation of hemoglobin (Hb) and unnecessary obliteration of red blood cells (RBCs). Thalassemia causes uneven degrees of sickness, which can broaden from massive to life-devastating. It is assessed that 1.5% of the world’s population are carriers of beta-thalassemia i.e. at any rate there are 80 million to 90 million individuals with an expected 60,000 new cases being conceived every year [9-10].

In offering counseling for inbreeding couples it is essential to recognize families with a known hereditary disorders and the family without disorders can be studied by family history and developing a pedigree [11-12].

Aims of the proposed study
1. To locate and identify families with hereditary disorders.
2. To study inbreeding among thalassemia affected families.
3. Counseling of the families affected with thalassemia disorders

Material and methods

Study Area

In the present study we mean to evaluate inbreeding in thalassemia influenced families resident in Tuman Leghari District D.G KHAN located in south of the Punjab, Pakistan.

A questionnaire was planned and conveyed in the study region to gather the applicable data amid the field review. The information gathering was finished in six months beginning from September 2015 to February 2016.

During the overview, 406 families were met for information accumulation. Population was isolated into three fundamental ethnic groups i.e. Leghari, Khosa, Saraiki other than Baloch.

Families Studied (Clinical Basis)
The families were found out by recognizing prob and amid the review for inbreeding. At that point the families were gone by at their places of home. The senior citizens and relatives of the families were met to get data about the thalassemia like hereditary issue and other important matters.

Statistical Analysis

Every one of the information was broke down by utilizing chi-square test as a part of a measurable programming bundle Mini Tab 17 rendition. The standard techniques and images portrayed by [13] were utilized for drawing family. What’s more, Cyrillic form 2.1.3 (Cherwell experimental distributed 1997, www.cherwell.com).

Results

During information accumulation, 406 families were drawn nearer arbitrarily to concentrate on the impacts of inbreeding. Ethically the most astounding positive reaction was found in Saraiki (other than Baloch) 47.30% than Leghari (32.26%) or Khosa (20.44%). Table 1 presents relative numbers and percent frequencies of families having a place with various ethnic groups and managerial ranges inside of the example. The Saraiki other than Baloch, Khosa and Leghari families were 47.30%, 32.26%, and 20.44%, respectively.

Table 1: Data summary on basis of tribes in Tuman Leghari Dera Ghazi Khan

| Ethnicity              | Number | Percentage |
|------------------------|--------|------------|
| Leghari                | 131    | 32.26      |
| Khosa                  | 83     | 20.44      |
| Saraiki Other than Baloch | 192   | 47.00      |
| Total                  | 406    |            |
Table 2: Statistical analysis for comparison of inbreeding and outbreeding marriages between Leghari, Khosa and Saraiki other than Baloch couples from Tuman Leghari.

| Samples                          | Inbreeding | Outbreeding | Total  | $X^2$  |
|----------------------------------|------------|-------------|--------|--------|
| Leghari                          | 126 (35.10%) | 05 (10.64%) | 131 (32.26%) | 10.87, $P < 0.001$ |
| Khosa                            | 67 (18.66%)  | 16 (34.04%)  | 83 (20.44%)  | 47.45, $P < 0.001$  |
| Saraiki Other than Baloch        | 166 (46.24%) | 26 (55.32%)  | 192 (47.00%) | 0.73, $P < 0.001$   |
| **Total**                        | 359 (88.42%) | 47 (11.58%)  | 406    | 34.05, $P < 0.001$ |

Table 3: Distribution of Marriage types in Ethnic groups of Tuman Leghari Tribe.

| Types of Marriages | F  | Leghari | Khosa | Non Baloch | Total  | $X^2$  |
|--------------------|----|---------|-------|------------|--------|--------|
| FC                 | 0.3821 | 49 (37.40%) | 36 (43.37%) | 72 (37.50%) | 157 (38.67%) | 8.57, $P < 0.001$ |
| SC                 | 0.1301 | 37 (28.24%) | 16 (19.28%) | 53 (27.60%) | 106 (26.11%) | 8.57, $P < 0.001$ |
| DR                 | 0.1085 | 40 (30.53%) | 15 (18.07%) | 31 (16.15%) | 86 (21.18%) | 8.57, $P < 0.001$ |
| NR                 | 0 | 05 (3.83) | 16 (19.28%) | 36 (18.75%) | 57 (14.04%)  | 8.57, $P < 0.001$ |
| **Total**          | 0.1354 | 131 (32.27%) | 83 (20.44%) | 192 (47.29%) | 406           | 8.57, $P < 0.001$ |

Table 4: Comparison of inbred and outbreeding marriages in Leghari, Khosa and Saraiki other than Baloch.

| Sample                          | Mean F | Inbreeding | Outbreeding | Total  | $X^2$  |
|---------------------------------|--------|------------|-------------|--------|--------|
| Leghari                         | 0.0301 | 126 (35.10%) | 05 (10.64%) | 131 (32.26%) | 10.87, $P < 0.001$ |
| Khosa                           | 0.0256 | 67 (18.66%)  | 16 (34.04%)  | 83 (20.44%)  | 47.45, $P < 0.001$ |
| Saraiki Other than Baloch       | 0.0303 | 166 (46.24%) | 26 (55.32%)  | 192 (47.00%) | 0.73, $P < 0.001$ |
| **Total**                      | 0.0287 | 359 (88.42%) | 47 (11.58%)  | 406    | 34.05, $P < 0.001$ |

Table 5: Inbreeding coefficient (F), inbreeding and outbreeding marriages with respect to husband’s education level.

| Population            | Mean F and Inbreeding | Husband’s education level | Total  | $X^2$  |
|-----------------------|-----------------------|---------------------------|--------|--------|
| Mean Inbreeding coefficient F | 0.0272 | 0.0292 | 0.0311 | 0.33, $0.90 < P < 0.75$ |
| **Total**             | 82 | 81 | 161 | 131 |

Table 6: Inbreeding coefficient (F), inbreeding and outbreeding marriages with respect to the Female’s educational level.

| Population          | Mean F and Marriage type | Female’s education level | Total  | $X^2$  |
|---------------------|--------------------------|--------------------------|--------|--------|
| Mean Inbreeding coefficient F | 0.0261 | 0.0279 | 0.0301 | 0.64, $0.90 < P < 0.75$ |
| **Total**           | 86 | 81 | 167 | 131 |

" 32 "
Table 7: Inbreeding coefficient (F), inbreed and outbreed marriages with respect to socioeconomic status of male.

| Population               | Mean F and Marriage type | Male’s Socioeconomic status | Total |
|--------------------------|--------------------------|-----------------------------|-------|
|                          |                          | Lower | Middle | Higher |       |
| Leghari                  | Inbreed                  | 0.0211 | 0.0256 | 0.0215 |       |
|                          | Total                    | 34    | 21     | 40     | 95    |
|                          | Outbreed                 | 17    | 13     | 06     | 36    |
|                          | Total                    | 51    | 34     | 46     | 131   |
| X²=8.55 P<0.001          |                          |       |        |        |       |
| Khosa                    | Inbreed                  | 0.0311 | 0.0301 | 0.0305 |       |
|                          | Total                    | 13    | 19     | 17     | 49    |
|                          | Outbreed                 | 09    | 11     | 14     | 34    |
|                          | Total                    | 22    | 30     | 31     | 83    |
| X²=1.02 P<0.001          |                          |       |        |        |       |
| Saraiki other than Baloch| Inbreed                  | 0.0256 | 0.0298 | 0.0227 |       |
|                          | Total                    | 23    | 37     | 48     | 108   |
|                          | Outbreed                 | 24    | 30     | 30     | 84    |
|                          | Total                    | 47    | 67     | 78     | 192   |
| X²=1.67 P<0.001          |                          |       |        |        |       |

Table 8: Inbreeding coefficient (F), inbreeding and outbreeding marriages with respect to the Female’s educational level.

| Population               | Mean F and Marriage type | Female’s socioeconomic status | Total |
|--------------------------|--------------------------|-------------------------------|-------|
|                          |                          | Lower | Middle | Higher |       |
| Leghari                  | Inbreed                  | 0.0231 | 0.0241 | 0.0253 |       |
|                          | Total                    | 27    | 19     | 39     | 85    |
|                          | Outbreed                 | 16    | 18     | 12     | 46    |
|                          | Total                    | 43    | 37     | 41     | 131   |
| X²=9.05 P<0.001          |                          |       |        |        |       |
| Khosa                    | Inbreed                  | 0.0256 | 0.0271 | 0.0278 |       |
|                          | Total                    | 13    | 23     | 19     | 55    |
|                          | Outbreed                 | 08    | 11     | 09     | 28    |
|                          | Total                    | 21    | 34     | 28     | 83    |
| X²=0.21 P<0.001          |                          |       |        |        |       |
| Saraiki other than Baloch| Inbreed                  | 0.0311 | 0.0321 | 0.0325 |       |
|                          | Total                    | 29    | 45     | 49     | 123   |
|                          | Outbreed                 | 15    | 36     | 18     | 69    |
|                          | Total                    | 44    | 71     | 67     | 192   |
| X²=6.28 P<0.001          |                          |       |        |        |       |

Table 9: Data on marriages in non-affected and affected families.

| S. N | Tribe                  | F.C | S.C | D.R | N.R | Total | X²  |
|------|------------------------|-----|-----|-----|-----|-------|-----|
| 1    | Leghari                | 44  | 37  | 32  | 50  | 118   | 11.67 |
| 2    | Khosa                  | 33  | 15  | 15  | 51  | 108   | 03.05 |
| 3    | Saraiki other than Baloch| 60  | 44  | 30  | 36  | 160   | 05.70 |
| 4    | Affected families      | 21  | 9   | 9   | 09  | 48    | 04.00 |

F.C = First Cousin, S.C = Second Cousin, D.R = Distantly related, N.R = Non-related
Table 10: Data on number of Thalassemia affected families.

| S.N | Tribe              | M/C | F/C | S.C | D.R | N.R | Total | X²  |
|-----|-------------------|-----|-----|-----|-----|-----|-------|-----|
| 1   | Leghari           | 03  | 01  | 08  | -   | -   | 11    | 9.66|
| 2   | Khosa             | 03  | 01  | 04  | 05  | -   | 14    | 2.86|
| 3   | Sareiki other than Baloch | 06  | 05  | 08  | -   | -   | 11    | 1.53|
|     | Total             | 12  | 06  | 08  | -   | -   | 26    | 11.69|

Table 11: Data on number of children in non-affected families

| S.N | Tribe      | 1st cousin | 2nd cousin | Distantly related | Non-related | Total | X²  |
|-----|------------|------------|------------|-------------------|-------------|-------|-----|
|     | M/F        | M/F        | M/F        | M/F               | M/F         |       |     |
| 1   | Leghari    | 77(33.35%) | 29(38.16%) | 27(39.13%)        | 50(31.85%)  | 151    | 25.8|
| 2   | Khosa      | 56(23.53%) | 13(17.11%) | 28(35.22%)        | 32(23.26%)  | 139    | 24.46|
| 3   | Sareiki    | 105(44.75%)| 34(44.75%) | 29(42.93%)        | 39(48.15%)  | 217    | 2.86|
|     | Total      | 238(49.77%)| 76(07.97%) | 69(07.24%)        | 81(08.5%)   | 516    |     |

Table 12: Data on children of affected families

| S.N | Tribe      | 1st cousin | 2nd cousin | Distantly related | Non-related | Total | X²  |
|-----|------------|------------|------------|-------------------|-------------|-------|-----|
|     | M/F        | M/F        | M/F        | M/F               | M/F         |       |     |
| 1   | Leghari    | 18(33.96%) | 05(17.24%) | -                 | -           | 23    | 7.13|
| 2   | Khosa      | 10(34.48%) | -          | -                 | -           | 16    | 3.72|
| 3   | Sareiki    | 19(35.85%) | 14(48.28%) | 21(100%)          | 08(41.97%)  | 53    | 30.94|
|     | Total      | 53(31.92%) | 29(17.47%) | 21(12.65%)        | 21(12.65%)  | 166   | 41.79|

Table 13: Data on children of thalassemia affected families

| S.N | Tribe      | 1st cousin | 2nd cousin | Distantly related | Non-related | Alive | Dead | Total | X²  |
|-----|------------|------------|------------|-------------------|-------------|-------|------|-------|-----|
|     | M/F        | M/F        | M/F        | M/F               | M/F         |       |      |       |     |
| 1   | Leghari    | 01(10.52%) | 02(11.76%) | -                 | -           | -     | -    | 03(10.56%) | 58    | 28.83|
| 2   | Khosa      | 05(26.32%) | 08(47.06%) | -                 | -           | -     | -    | 13(17.11%) | 26(17.00%) | 44.03|
| 3   | Sareiki    | 12(63.16%) | 07(18.18%) | 04(100%)          | -           | -     | -    | 16(18.63%) | 68(44.44%) | 10.65|
|     | Total      | 19(12.6%)  | 17(11.11%) | 11(07.19%)        | 04(02.61)   | 04(02.41) | 166   | 53.51|

Table 14: Awareness about Thalassemia

| Genetic | Treatable | Not treatable | Medicinal effect | Curse | Superstitious | Ignore |
|---------|-----------|---------------|------------------|------|---------------|--------|
| 290     | 37(71.43%)| 37 (9.11%)    | 27 (6.65%)       | 10   | 2.46%         | 08     |
|         |           |               |                  | 04   | 0.99%         | 30     |

Table 15: Behavior of the Parents, Relatives and Society

| Behavior Type | Parents | Relatives | Society |
|---------------|---------|-----------|---------|
| Good          | 218     | 191       | 187     |
| Bad           | 43      | 71        | 72      |
| Ignore        | 120     | 123       | 127     |
| Mercy         | 25      | 21        | 20      |
| Total         | 406     | 406       | 406     |

For the study displayed here, 406 families are incorporated. In which twenty six families/couples are influenced with thalassemia.

**Thalassemia Affected families**

Twenty six with thalassemia were learned from various union councils of Tuman Leghari of Dera Ghazi Khan District. Eleven Families/couples has a consign with Leghari, Khosa and Eleven to Sareiki other than Baloch.

![Fig 1: Some Thalassemia Affected individuals in Tuman Leghari](image)
Clinical Features of Thalassemia

In above mentioned families affected with Thalassemia the symptoms observed related to growth problems – not putting on weight or growing in height. Anaemia – red blood cell deficiency, leading to tiredness, weakness and shortness of breath. Jaundice – yellowing of the skin and whites of the eyes. Regular transfusion of blood from donors.

Discussion

In this study, a questionnaire was distributed to distinguish different types of marriages, for examples, particularly the inbreeding marriages and to evaluate its effect on the population of Tuman Leghari resident of D. G. Khan District of Punjab Province. In the present study, our outcomes furthermore demonstrated that 70.52% marriages in the overall population and relational unions in all inclusive community respectively 35.10%, 18.66%, and 46.24% are inbred (Table.2 and 3). Inside of the limits of inbreeding characterized by the above parameters, close inbreeding unions are particular because of a coinciding of key elements of groups and individual-level foundation essentials. This study revealed a high rate of inbreeding 88.42% with 0.0201 mean coefficients of inbreeding by and large population (Table.4) our outcomes are in accord with the results of a past study aimed at Pakistan level [14]. Some of the possible reasons of high frequency of inbreeding may be:

1. Tribalism is intensely recognized in progress account of population understudy.
2. High rate of inbreeding alongside the variables like geological conditions, antagonistic method for transportation, destitution.
3. More distant family culture presence in the range expands shot of inbreeding.

This study revealed a high rate of inbreeding in ethnic population like Leghari (35.10%), Khosa (18.66%) and Saraiki other than Baloch (46.24%) (Table.4.4). These ethnic gatherings varied essentially from each other regarding inbreeding relational unions. We concentrated on the impact of guidance (both male and female), male financial status, and male occupation on predominance of inbreeding. Rate of inbreeding is as a result firmly connected with these components (Table.5 and 6).

In the present study, the impact of marriage sorts on particular gathering of hereditary disorder thalassemia was additionally examined. General inbreeding relational unions were discovered altogether most inexhaustible among burdened couples when contrasted with overall public (Table.7 and 8). (Table.9 to 10). Awareness about the hereditary disorders were requested in the study from every individual from family demonstrates the ailments were considered firmly associate to the deadly hereditary qualities allowed from solid inbreeding among families with rate of 71.43% when contrasted with treatable (9.11%), not treatable (6.65%), Medicinal impact (2.46%), curse (1.97%), superstitious (0.99%) and overlook (7.39%). Conduct of the folks, relative and society were additionally addressed from families which communicated a higher rate of good conduct with the tormented youngsters 53.69, 47.04 and 46.06 individually. Disregard of influenced youngsters was additionally high in rate among benevolence and terrible demeanor of parents, relatives and society (Table 11 to 15).

References

1. Gadgil M, Joshi NVC, Manoharan S, Patil S, Prasad UVS. Peopling of India; in Balasubramanian D, Rao NA. The Indian Human Heritage. Hyderabad, Universities Press, 1998, 100-129.
2. Amin-ud-din M, Haque S, Ahmad W. Atrophia with papular lesions in two Pakistani consanguineous families resulting from mutations in the human hairless gene. Arch Dermatol Res. 2005; 297:226-230.
3. Reddy VR, Rao AP. Effects of parental consanguinity on fertility, mortality, and morbidity among the pattuasalis of Tirupati, South India. Hum Hered. 1978; 28:226-234.
4. Shami SA. Consanguineous marriages in Mianchannu and Muridke (Punjab) Pakistan. Biologia. 1983; 29:19-30.
5. Minhas IB. effects of consanguineous marriages on offspring mortality in the City of Jhelum (Punjab), Pakistan. Biologia. 1984; 30:153-165.
6. Shami SA, Schmitt LH, Bittles AH. Consanguinity-related antenatal and postnatal mortality in the populations of seven Pakistani Punjab cities. J Med Genet. 1989; 26:267-271.
7. Shami SA, Siddiqui H. The effects of parental consanguinity in Rawalpindi City (Punjab), Pakistan. Biologia. 1984; 30:189-200.
8. Asien P, Listowsky I. Iron transport and storage proteins. Ann. Rev. Biochem. 1980; 49:357-393.
9. Angelucci ED, Barocianci Lucarelli G, Baldassari M, Galimberti M, Giardini C. Needle liver biopsy in thalassaemia analyses of diagnostic accuracy and safety in 1184 consecutive biopsies. Br. J Haematol. 1995; 89:757-761.
10. Baig SM, Azhar A, Hassan H, Baig JM, Aslam M. Prenatal diagnosis of beta thalassaemia in Southern Punjab. Prenat. Diagn. 2006; 26(10):9035.
11. Pippard, Powell LW. Iron metabolism in health and diseases. Philadelphia: WB Saunders, 1994, 63-95.
12. Shaw NJ, Impact of disordered puberty on bone density in beta Thalassaemia Major. Br. J Haematol. 2003; 120:353-358.
13. Brittenham GM, Cohen AR, McLaren C, Martin MB, Griffith MM, Nieuwhuys AW. Hepatic iron stores and plasma ferritin concentration in patients with sickle cell anemia and thalassemia. Am. J Hematol. Med. 1993; 42:81-85.
14. Cooley TB, Lee P. A series of cases of splenomegaly in chicken anemia and peculiar changes. Trans. Am.
15. Dabbagh AJ, Manion T, Lynch SM, Frei B. The effect of iron overload on plasma and liver antioxidants. Biochem. J. 1994; 300:799-803.

16. Angelucci. Patterns of iron distribution in liver cells in beta thalassemia studied by X-ray microanalysis. Haematologica. 2002; 87(5):479-484.