DNA flow cytometry of left and right testes in normospermic patients affected by left varicocele

Guglielmo Bonanni¹², Alessandra Calcagno¹, Giacomo Mammana², Enzo Chemello², Nicola Pennelli³ and Ismaele Mastrogiacomo⁴

¹Institute of Semeiotica Medica, University of Padova, Via Ospedale 105, ²Department of Surgery, Military Hospital, Via Giovanni da Verda and ³Institute of Pathology, University of Padova, Via Gabelli 61, 35100 Padova Italy
⁴To whom correspondence should be addressed

Introduction

Varicocele is well known to be the most frequent cause of male infertility (Dubin and Alemar, 1975). Many men affected by this disease may, however, still be able to procreate without therapy (Clarke, 1966) due to the resistance of some subjects to the toxic effect on spermatogenesis and/or because the varicocele induces gradual and progressive damage. Fertility may therefore be preserved in the early stages of the condition.

Studies have demonstrated that induced varicocele in both rats (Nagler et al., 1985) and dogs (Saypol et al., 1981) provokes alterations in spermatogenesis which become more marked over a period of time.

The same behaviour was described in men by Cheval and Purcell (1992), who studied 13 normospermic men aged 25–35 years with a palpable varicocele which was left untreated. After an average interval of 44 months, these subjects were found to have an 80% reduction in sperm number. In 1993, Gorelick and Goldstein showed that varicocele was present in 35% of infertile men who had never been fertile (primary infertility) and in 81% of infertile subjects who had previously been able to father a child (secondary infertility). From the evidence of a progressive reduction in fertility Gorelick and Goldstein (1993) suggested that preventive surgical treatment is indicated in youth.

The effectiveness of varicocelectomy is still debatable: several authors (Yamamoto et al., 1994; Nieschlag et al., 1995) found no statistically significant difference in pregnancy rates between treated and non-treated varicocele groups. The possible explanations are either that varicocelectomy does not remove the cause of the varicocele-induced infertility or that the testicular alterations are irreversible.

It is logical to assume, however, that young subjects affected by varicocele have mild spermatogenic impairment which is likely to be more marked in the testicle ipsilateral to the varicocele, as indicated by the common clinical finding of reduced testicular volume on this side (Lipshultz and Corriere, 1977; Pozza et al., 1983).

To verify this hypothesis, fine needle (21 G) aspiration biopsy (FNAB) of testes was performed on young normospermic varicocele subjects. This procedure provides sufficient material to evaluate spermatogenesis (Foresta and Vannotti, 1992; Verma et al., 1992) by flow cytometry which automatically and accurately identifies the different testicular cells (haploid, diploid and tetraploid) (Hellstrom and Kaack, 1990; Hittmair et al., 1992). This method is therefore suitable for a comparative analysis of the right and left testicles.

Materials and methods

Twenty-six normospermic soldiers (sperm density >20×10⁶/ml), aged 19–21 years, suffering from left varicocele were studied. All
Flow cytometry in left varicocele

Table I. Seminal parameters in varicocele patients and control group. VCL, curvilinear velocity (µm/s); VSL, straight line velocity (µm/s); ALH, amplitude of lateral head displacement (µm).

<table>
<thead>
<tr>
<th></th>
<th>Number ×10</th>
<th>Motility %</th>
<th>Morphology %</th>
<th>VCL (µm/s)</th>
<th>VSL (µm/s)</th>
<th>Linearity %</th>
<th>ALH (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varicocele group ± SD</td>
<td>46.4</td>
<td>20.3**</td>
<td>49.8**</td>
<td>77.2</td>
<td>31.6**</td>
<td>46.3</td>
<td>2.7**</td>
</tr>
<tr>
<td></td>
<td>40.2</td>
<td>6.8</td>
<td>7.8</td>
<td>19.2</td>
<td>8.5</td>
<td>10.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Control group ± SD</td>
<td>71.8</td>
<td>30.7</td>
<td>65.9</td>
<td>87.2</td>
<td>37.9</td>
<td>46.7</td>
<td>3.6</td>
</tr>
<tr>
<td></td>
<td>24.6</td>
<td>7.4</td>
<td>7.8</td>
<td>19.6</td>
<td>9.1</td>
<td>10.5</td>
<td>0.84</td>
</tr>
</tbody>
</table>

**P < 0.001 when compared to control group.

Results

Seminal parameters in the varicocele subjects and in the control group are shown in Table I. Although sperm concentration was similar in the two groups, the percentage of motile spermatozoa was lower in those affected by varicocele, since asthenospermia was an indication for varicocelectomy. The values of VSL, ALH and percentage of morphologically normal spermatozoa were significantly lower in the varicocele group than in the controls. VCL and linearity did not show any statistically significant difference between the two groups.

Flow cytometric analysis revealed four peaks in the nuclear DNA cellular content: (i) two peaks for haploid cells (1-A), the first composed of highly condensed nuclear cells (1-Ac), essentially spermatozoa, and the second of less condensed cells, essentially spermatids (1-Anc); (ii) a third peak of diploid cells (2-D): somatic cells, G1-stage spermatogonia, primary and secondary spermatocytes, and (iii) a fourth peak of tetraploid cells, essentially post-leptotene primary spermatocytes and G2–M-stage spermatogonia (4-T) (Spanò and Evenson, 1993). In six samples it was possible to distinguish between condensed and non-condensed haploid cells and thus they appear as only one peak.

Flow cytometry showed the left testis to have a lower percentage of haploid cells (1-A, condensed and non-condensed cells) (Figure 1) than the right (mean 48.4 ± 17.9 versus 57% ± 15.4, P < 0.05). Where condensed and non-condensed cells could be differentiated, significantly fewer condensed cells were found on the left side than on the right (respectively 19.7 ± 11.2 versus 31.5 ± 13.5%, P < 0.004), whereas there was no statistically significant difference in respect of the percentages of either non-condensed cells (26.6 ± 14.8% on the left and 25.9 ± 10.3% on the right) and tetraploid cells (11.3 ± 5.6% on the left and 12.4 ± 6.2% on the right). The diploid cell percentage was significantly higher in the left testis than in the right (37.0 ± 18.5 versus 25.5 ± 9.6, P < 0.003).

Flow cytometric analysis of the cadaver testicular biopsy tissue did not show any statistical difference between the left and right testes in respect of the percentages of haploid, diploid and tetraploid cells (Figure 2).

Discussion

The testicular histological picture in varicocele patients has been investigated repeatedly with very different descriptions. The most frequent finding seems to be the presence of immature germ cell exfoliation in the tubular lumen (Cameron et al., 1980; Jones et al., 1988) but a reduction in germinal cells has also often described (Charney, 1962). Rarely, these cells are completely absent (Wang et al., 1991). Different stages of maturational arrest (McFadden and Mehan, 1978) and changes in Sertoli cells may also be seen (Cameron and Snydle, 1982). The lower the sperm density, the greater the change in the pathogenesis of varicocele (Agger and Johnsen, 1978; Hadziselimovic et al., 1986).

Few studies have compared the histological features of the testis ipsilateral to the varicocele with those of the contralateral testis. It is well known that subjects with severe oligospermia have histological changes in both testes and researchers who examine the testicular histology by traditional (Charney, 1962; Hadziselimovic et al., 1986) or semiquantitative methods (Agger and Johnsen, 1978; Wang et al., 1991) usually find no differences between the testes. Although more marked changes in the ipsilateral testes have been described in pubes-
cent (<16 years) varicocele patients (Pozza et al., 1983; Kass et al., 1987), these findings conflict with the data of other studies (Jones et al., 1988).

As FNAB for flow cytometric analysis could not be performed on healthy subjects for obvious ethical reasons, there is no control group for a comparison of the results obtained in our varicocele subjects. The cadaver data could not be used as controls for the percentages of the peaks because the subjects were much older than those studied and the tissue was obtained by surgical excision rather than fine needle biopsy. It is, however, noteworthy that in subjects with no monolateral testicular disease, the cytometric analysis of the right testis is similar to that of the left.

According to the literature (Hittmair et al., 1992), the percentage of haploid cells in the right testis (57%) can be considered normal. This result was expected as our subjects had been chosen for their normal sperm concentration.

The finding of a significantly higher percentage of haploid cells in the right testis than in the left is remarkable. As haploid cells appear after meiosis and represent the last stage of spermatogenesis, their reduced number indicates impairment of spermatogenesis in the testis ipsilateral to the varicocele.

The pathogenic mechanism of varicocele-induced infertility is still doubtful. Increased temperature (Yamaguchi and Sakatoku, 1989) and blood reflux from the kidneys or adrenal glands (Comhaire and Vermeulen, 1974) have been suggested as causes of damage to the testis ipsilateral to the varicocele. The changes in the ipsilateral testis probably occur at an earlier stage and are more marked than those in the contralateral testicle.

Flow cytometric analysis has not been utilized in the studies of varicocele in men, although it has been used in the study of induced varicocele. Nagler et al. (1985) found that varicocele in rats did not alter the flow cytometric picture until day 25 after surgery. On day 49, a decrease in haploid cells and increase in diploid cells occurred in the left testis only. Takihara et al. (1990) described the same pattern in both testicles of rats, the changes being more evident in the left testis. On the whole, therefore, the experimental data are compatible with our findings.

It is important to establish the characteristics of the variations in haploid and diploid cell percentages as these changes appear to constitute the initial varicocele-induced damage. The reduction of haploid cells on the left side particularly affects

![Figure 1](image1.png)

**Figure 1.** Flow cytometric analysis of right and left testis in varicocele patients. 1-Ac, condensed haploid cells; 1-An, non-condensed haploid cells; 1-A, total haploid cells; 2-D, diploid cells; 4-T, tetraploid cells. Values are expressed as mean ± SD.

![Figure 2](image2.png)

**Figure 2.** Cytometric analysis of right and left testis in nine cadavers. 1-A, haploid cells; 2-D, diploid cells; 4-T, tetraploid cells. Values are expressed as mean ± SD.
condensed cells which are significantly fewer than in the right testis. The non-condensed cell percentages are similar in both testicles.

Nuclear chromatin condensation and transformation of elongated spermatid to spermatozoa usually occur contemporaneously during spermiogenesis. The reduced percentage of condensed cells indicates impairment of the last stage of spermiogenesis which could be due to either a reduced number of spermatozoa or to sperm immaturity caused by an absence of chromatin condensation.

The existence of this type of spermatogenic impairment would seem to be confirmed by the frequent histological finding of germinal cell exfoliation into tubular lumen. If germinal cells are deprived of the nutritive and stimulating influence of the Sertoli cells, complete germ cell maturation does not usually occur (Russell and Griswold, 1993).

The increase in diploid germ cells, which are essentially spermatogonia, may be explained by the presence of a maturational block at different steps of spermiogenesis. A maturational arrest at the spermatogonia stage would decrease the number of all the cells which are derived from spermatogonia, i.e. tetraploid cells; the percentages of these latter cells in both testes of the subjects studied were, however, found to be similar.

The higher percentage of diploid cells may therefore be indicative of hypospermatogenesis, characterized by a global reduction in germ cells and by a relative increase in the percentage of Sertoli cells.

The abnormal percentages of haploid and diploid cells in the left testis of our normospermic varicocele subjects is a significant clinical finding in that it confirms that varicocele-induced testicular damage may be present in males whose sperm concentration is still within normal limits. It would appear that both testicles must be damaged before a reduction in sperm concentration is seen, as subjects who have damage to the left testis only may be normospermic.

Acknowledgements

This study was supported by a grant from CNR, Rome, Italy, no. 94.02767.CT0.

References


