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"Sterilità di coppia, poliabortività e correlazione con alterazioni dell'emostasi""

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Titolo della tesi

Sterilità di coppia, poliabortività e correlazione con alterazioni dell'emostasi.

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Abstract

Background - Thrombophilia is a well known risk factor for female infertility. Several studies are available in the Literature since 1980's underlying the relations between inherited and\or acquired thrombophilia and recurrent pregnancy loss. Yet, recent studies underlined a possible relationship between inherited thrombophilia and repeated in vitro fertilization failures. On the other hand acquired thrombophilia is induced by hormonal treatment based on oral contraceptive use or hormonal treatment directed to controlled ovarian hyperstimulation.

Aim - The aim of this study is to demonstrate the role of thrombophilia in patients affected by unexplained sterility and unexplained recurrent pregnancy loss and in the daily clinical management of patients ongoing screening for female sterility.

Patients and methods - We selected 60 patients directed to our outpatient for clinical management of female infertility. 40 patients was affected by recurrent foetal loss, while 20 patients was referred for unexplained female sterility. As control group we selected 30 subjects without thrombotic episodes in their anamnesis and with one or more successful pregnancy and without gestational complication or miscarriage.

All subject were screened for molecular thrombophilia due to inherited defects (i.e. factor V Leiden gene variant, prothrombin A20210G gene variant and MTHFR C677T gene variant, protein S deficiency, protein C deficiency, AT III deficiency) or acquired causes (i.e. acquired protein C resistance other than factor V Leiden gene variant, hyperhomocysteinemia other than MTHFR

C677T gene variant, increased factor VIII, reduced factor XII, antiphospholipid syndrome).

Results - We reported 2 cases report in which the role of acquired thrombophilia due to oral contraceptive use trigger a superior mesenteric vein thrombosis and another one in which an acute carotid thrombosis was triggered by ovarian hyperstimulation syndrome. On the other hand a review in which the role of thrombophilia in women affected by recurrent pregnancy loss was performed and 2 editorial comments was provided. One of them was based on therapeutic role of antithrombotic treatment based on low molecular weight heparin in thrombophilic women affected by recurrent pregnancy loss. The second one explained the state of the art for the diagnosis of ovarian vein thrombosis. Moreover we identified a possible role of d-dimer for the fast identification of thrombophilia in women ongoing a screening for infertility. Furthermore, the role of hyperhomocysteinemia not only in women affected by recurrent pregnancy loss but also in women affected by unexplained female sterility was described for the first time in our data.

Conclusion - In conclusion our data confirmed several aspect of inherited, acquired and combined thrombophilia in several clinical settings of women ongoing screening of infertility but open also a new perspectives for the daily clinical management of this kind of patients in particular for the screening of hypercoagulable state and hyperhomocysteinemia. These data may be relevant in next years also for possible therapeutic aspects in this clinical setting.

Indice

Parte 1 – Premessa.

1. Sterilità di coppia.

La **sterilità di coppia** è quella condizione in cui vi sia assenza di concepimento dopo un anno di rapporti sessuali frequenti e non protetti (1).
In tale definizione viene sottolineata l'importanza di due fattori: il tempo e la frequenza coitale. Per quanto riguarda il limite temporale di almeno un anno, studi sulla fertilità naturale della specie umana hanno dimostrato come, anche in assenza di condizioni patologiche, la probabilità di ottenere un concepimento che esiti nella nascita di un bambino vivo, entro un ciclo mestruale, sia solo del 25%; probabilità che aumenta fino al 72% dopo sei mesi, e che diviene pari all'80-90% dopo un anno (1). Per quanto riguarda la frequenza coitale, è stato dimostrato come la probabilità di concepimento sia pari al 15% nelle coppie che abbiano rapporti con frequenza settimanale e dell'83% nelle coppie con quattro o più rapporti settimanali (1).
Vale infine la pena ricordare come anche la variabile età (in particolar modo quella femminile) incida significativamente sulla sterilità di coppia. Innumerevoli studi hanno dimostrato in modo inequivocabile come vi sia una riduzione statisticamente significativa della fertilità femminile con l'avanzare dell'età e come il trentacinquesimo anno di età rappresenti per la donna un limite oltre il quale si verifica una irreversibile e progressiva riduzione della fertilità.
Si è soliti parlare di **sterilità primaria** quando la coppia non ha mai ottenuto una gravidanza, di **sterilità secondaria** quando la coppia ha riportato almeno una gravidanza a termine. Infine, per **infertilità**, si intende l'incapacità di proseguire la gravidanza fino ad un'epoca di vitalità del feto (2-3).

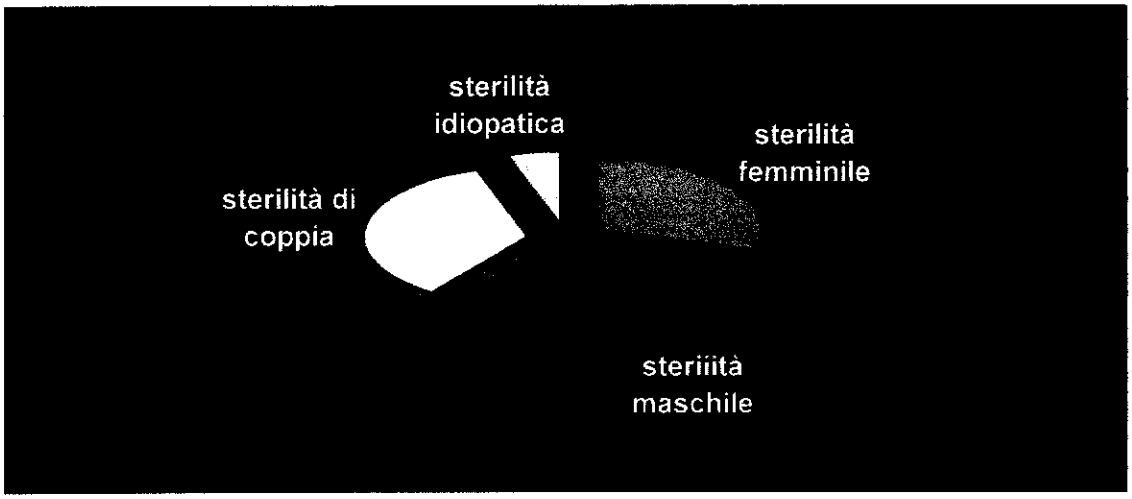
1.1. Epidemiologia della sterilità di coppia

Dal punto di vista epidemiologico, si ritiene che attualmente oltre il 15% delle coppie sia affetto da problematiche di sterilità o di infertilità. Negli ultimi 20 anni il tasso di coppie sterili ha subito un incremento notevole.

Una delle principali motivazioni che ha contribuito a tale aumento è da ricercarsi nella tendenza sempre più frequente a procrastinare l'età del matrimonio, laddove, come precedentemente detto, la fertilità (in particolar modo quella femminile) si riduce sensibilmente e progressivamente con l'età (1).

1.2. Cause di sterilità

Le cause della sterilità possono essere legate ad una condizione patologica della donna, dell'uomo o di entrambi. Pertanto si distinguono: una **sterilità femminile** (35% dei casi), una **sterilità maschile** (35% dei casi) ed una **sterilità di coppia** (30% dei casi). Tuttavia, in alcuni casi non è possibile identificare alcun fattore capace di interferire con la capacità riproduttiva della coppia; si parla in tali casi di **sterilità idiopatica o da causa inspiegata** (5% dei casi) (1).



1.3. Cause di sterilità femminile.

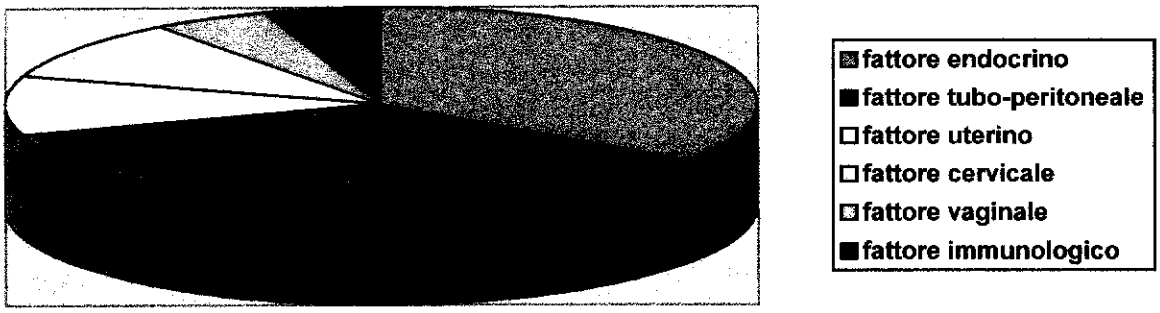
La sterilità femminile può essere dovuta ai seguenti fattori:

- **Alterazioni endocrine** (30-40% casi): comprendono tutte quelle condizioni in cui vi sia la mancanza dell'ovulazione (sindrome dell'ovaio policistico, iperprolattinemia, distiroidismi, diabete mellito), insufficienza del corpo luteo e quelle affezioni (disendocrinie extra-genitali e

dismetaboliche) capaci di interferire con la funzionalità dell'asse ipotalamo-ipofisi-ovaio (sindrome di Cushing, ipo- e iper-tiroidismo) (1-2).

- **Alterazioni vaginali** (5%): alterazioni anatomiche (malformazioni vaginali quali agenesia, presenza di setti e stenosi), infiammatorie e infettive (vaginiti) e funzionali (vaginismo e dispareunia) (1-2).
- **Alterazioni immunologiche** (1-5%): alterazioni della risposta immunitaria nell'ambito della coppia possono interferire con la fertilità a vari livelli: transito degli spermatozoi lungo le vie genitali femminili; fecondazione; fasi precoci dello sviluppo embrionale. Le forme più frequenti sono caratterizzate dalla presenza di anticorpi rivolti contro antigeni spermatici sia nel liquido seminale (auto-immunizzazione maschile), sia nel sangue e nel muco cervicale della partner (iso-immunizzazione femminile) (1-2).
- **Alterazioni tubo-peritoneali** (35% casi): comprende le alterazioni anatomiche e funzionali a carico delle tube e le modificazioni di natura fisica, chimica o meccanica che si realizzano all'interno della cavità pelvica. Rientrano in tale categoria la patologia tubarica primitiva (processi infiammatori acuti e cronici, malformazioni), l'endometriosi e tutte quelle condizioni responsabili della formazione di aderenze peritubariche che, dislocando e comprimendo le tube, ne compromettono la funzionalità (la stessa endometriosi, infiammazioni degli organi pelvici, pregressi interventi laparotomici, precedenti gravidanze ectopiche) (1-2).
- **Alterazioni uterino-endometriali** (5-10%): comprende le malformazioni dell'apparato genitale (agenesia uterina, utero setto, utero bicorne, ecc.), i processi infiammatori a carico dell'endometrio (endometriti), le patologie endouterine (miomi e polipi endometriali che si accrescono nella cavità uterina; presenza di processi aderenziali che alterano la cavità uterina) (1-2).
- **Alterazioni cervicali** (5-15%): alterazioni anatomiche (ipoplasia e stenosi), infiammatorie ed infettive (cerviciti) e funzionali (carenza muco cervicale) della cervice uterina possono determinare una inadeguata

interazione tra il seme del partner maschile e il microambiente del tratto genitale femminile a livello cervicale (1).



2. Abortività ricorrente.

Per **abortività ricorrente** si intende la presenza anamnestica di due o più episodi abortivi spontanei consecutivi in una coppia, secondo alcuni Autori, o di tre o più aborti consecutivi secondo altri. Comunemente il termine viene oggi utilizzato in entrambe le accezioni (4). La ricerca di eventuali cause di aborto si impone già dopo il secondo aborto. La ricerca con metodiche diagnostiche è infatti raccomandata dopo ripetuti episodi abortivi con epoca gestazionale < 10 settimane oppure già dopo un unico episodio abortivo con età gestazionale > 10 settimane. I dati della Letteratura indicano che il rischio di ripetizione di un episodio abortivo dopo un primo evento è pari al 13.5%, ma aumenta al 24% dopo 2 episodi e al 33% dopo tre eventi (4).

Dal punta di vista eziopatogenetico possiamo distinguere diversi fattori di rischio, tuttavia in un'alta percentuale di casi la causa della abortività ripetuta rimane inspiegata (4).

Si possono distinguere cause genetiche, cause endocrine, cause anatomiche, cause infettive, cause immunologiche / infiammatorie croniche, cause ematologiche con alterazioni specifiche dell'emostasi.

2.1. Cause di Abortività ricorrente.

- **Cause genetiche.** L'incidenza delle cause genetiche varia nelle diverse casistiche con percentuali variabili sino al 40-60% (5). Possiamo distinguere anomalie cromosomiche quali trisomie, aneuploidie, monosomie, aberrazioni strutturali e specifiche alterazioni geniche (polimorfismi genetici); nuovi dati disponibili nella Letteratura inoltre, sottolineano un potenziale ruolo causale di "imprinting genomico" e il mosaicismo del cromosoma X (4).
- **Cause anatomiche**. L'incidenza delle cause anatomiche varia nelle casistiche con percentuali variabili tra il 10% e 30%. Possiamo anche distinguere diverse condizioni quali anomalie anatomiche congenite con malformazioni uterine (utero unicorne, utero setto, utero didelfo, utero bicorne) e anomalie acquisite (incontinenza cervicale, sinechie endouterine, leiomiomi sottomucosi) (4).
- **Cause endocrine.** L'incidenza delle cause endocrine varia nelle casistiche con percentuali variabili tra il 10% e 20% (5). Possiamo riconoscere cause endocrine di abortività ripetuta quali deficit di fase luteale, iperprolattinemie, sindrome dell'ovaio policistico, distiroidismi, diabete mellito metabolicamente non compensato) (4).
- **Cause infettive**. L'incidenza delle cause infettive varia nelle casistiche con percentuali intorno al 5%. Attualmente un ruolo patogenetico sembra essere riconosciuto a infezioni da Chlamydia trachomatis, Ureaplasma urealyticum e Mycoplasma spp (5).
- **Cause immunologiche**. L'incidenza delle cause immunologiche varia nelle casistiche con percentuali molto variabili. Dal punto di vista patogenetico tali patologie inducono processi flogistici cronici con possibile coinvolgimento autoimmunitario. Le patologie più comunemente coinvolte nelle poliabortività sono il Lupus eritematoso sistemico, la sclerosi sistemica, sindromi da anticorpi antifosfolipidi secondarie a patologia autoimmunitaria, morbo celiaco. E' stata inoltre segnalata da diversi Autori la possibilità di episodi abortivi in donne con

isolata positività degli anticorpi antinucleo (ANA) con titolazione quantitativa variabile (4).

- **Cause psicologiche**. L'incidenza delle cause psicologiche è estremamente variabile non essendo tutt'ora accettata da tutti i gruppi di Ricerca.
- **Cause ambientali**. Anche l'incidenza delle cause ambientali è estremamente variabile e tutt'ora non accettata da tutti i gruppi di Ricerca. Potenziale ruolo è stato attribuito a fumo di sigaretta, utilizzo di farmaci, abuso di caffè, alcool e sostanze psicoattive.
- **Cause ematologiche**. Nell'ultimo ventennio i dati della letteratura hanno mostrato un ruolo patogenetico specifico nella abortività ricorrente delle alterazioni dell'emostasi con tendenza alla trombofilia e all'ipercoagulabilità. Anche qui l'incidenza di tali alterazioni è estremamente variabile nelle casistiche disponibili nella Letteratura per differenti criteri di inclusione e esclusione delle pazienti studiate.

3. La trombosi e la trombofilia.

Mentre è noto da secoli che difetti congeniti della coagulazione del sangue causano malattie emorragiche come l'emofilia, è solo da qualche decennio che si conoscono cause ereditarie di trombosi (trombofilia ereditaria).

La manifestazioni cliniche della patologia trombotica si possono presentare sia a livello dei vasi venosi (trombosi venosa) che dei vasi arteriosi (trombosi arteriosa).

La trombosi può verificarsi in assenza di cause scatenanti (primitiva o idiopatica) o in presenza di fattori di rischio (secondaria) (6,7).

La patologia trombotica venosa ha un'incidenza di 1/1000 persone /anno nella popolazione generale dei paesi occidentali e una prevalenza di 1/100 persone/anno nella popolazione selezionata per nuovi eventi clinici di trombosi venosa. Tale frequenza è pari alla frequenza di patologia trombotica arteriosa sebbene questa stessa presenti anche altri fattori di rischio (ipertensione arteriosa, fumo di sigaretta, età, dislipidemie, diabete mellito)

che alterano la parete vascolare facilitando il processo aterosclerotico e quindi l'aterotrombosi.

Per tali motivi la patogenesi delle trombosi eredofamiliari viene comunemente associata alla patogenesi della trombosi venosa sebbene siano note manifestazioni trombotiche eredofamiliari del versante arterioso. Numerosi studi infatti sono soliti riportare e sottolineare il ruolo dei fattori di rischio trombotico prevalentemente per la trombosi venosa, sebbene questi stessi siano coinvolti anche nella patogenesi della trombosi arteriosa.

L'osservazione di episodi tromboembolici venosi ricorrenti in alcune famiglie, soprattutto in età giovanile in soggetti della medesima famiglia ha indotto ad ipotizzare l'esistenza di uno stato di trombofilia.

La **trombofilia ereditaria** viene definita come la tendenza, determinata da cause genetiche, alla trombosi venosa, che tipicamente è caratterizzata dalla familiarità, dalla comparsa di eventi in età giovanile (prima dei 40-45 anni), dalla mancanza di altre cause e dalla tendenza a recidivare. Come già detto i pazienti affetti da trombofilia possono, sia pure più raramente, sviluppare anche episodi di trombosi arteriosa.

Dal punto di vista patogenetico la trombosi è il risultato di un'alterazione dell'equilibrio tra forze protrombotiche e antitrombotiche a livello del flusso sanguigno, e di un disturbo dell'interazione tra sangue e parete endoteliale. Già nel 1856 R.Virchow (8) diede la prima definizione patogenetica della trombosi descrivendo i tre eventi di perturbazione dell'omeostasi vascolare responsabili della formazione del trombo:

1. alterazione del flusso sanguigno (stasi o turbolenza)
2. alterazione dello stato di coagulabilità del sangue (ipercoagulabilità)
3. danno della parete vasale (endotelio)

Gli studi di biologia cellulare, di biochimica e di biologia molecolare hanno migliorato e approfondito le nostre conoscenze in merito alla patogenesi degli eventi trombotici, ma hanno confermato i capisaldi della triade di Virchow, che risulta valida tutt'oggi.

Sebbene le trombosi arteriose e quelle venose abbiano una patogenesi lievemente diversa, in entrambe è comune uno stato di squilibrio nel sistema della coagulazione con una conseguente **ipercoagulabilità** (9-12).

Il sistema della coagulazione è un complesso enzimatico costituito da una cascata di attivazione di zimogeni inattivi, regolato da cofattori e da inibitori, che giocano un ruolo fondamentale nell'emostasi, indispensabile per il mantenimento dell'integrità vascolare e per un corretto flusso sanguigno all'interno dei vasi.

Tradizionalmente si è soliti indicare 2 differenti vie di attivazione della coagulazione:

- la via della fase di contatto o via intrinseca
- la via del complesso fattore VII attivato-fattore tissutale o via estrinseca.

Studi sul meccanismo di attivazione "in vivo" della coagulazione hanno dimostrato che la via del complesso fattore VII attivato-fattore tissutale è la principale via di generazione del fattore X attivato (fattore Xa). Il complesso fattore X attivato-fattore tissutale infatti può attivare sia direttamente che indirettamente il fattore X, mediante proteolisi del fattore IX, saltando i fattori del sistema di contatto. La trombina così generata può attivare a sua volta i fattori XI, IX e VIII, promuovendo un'amplificazione della cascata della coagulazione con la proteolisi del fibrinogeno in fibrina (13-15).

Il sistema della coagulazione è, a sua volta, regolato da un sistema di elementi ad attività anticoagulante, come la antitrombina III, il sistema proteina C-proteina S e il sistema di inibizione del fattore tissutale. Il ruolo esercitato da tali proteine è quello di autoregolare l'attivazione della cascata coagulativa per evitare stati di ipercoagulabilità. La perturbazione dell'equilibrio tra questi elementi a funzione anticoagulante e i fattori ad azione procoagulante con uno sbilanciamento a favore degli elementi procoagulanti determina uno stato di ipercoagulabilità.

Negli ultimi anni sono state identificate numerose alterazioni molecolari dell'emostasi responsabili dello stato di trombofilia e della conseguente ipercoagulabilità (tabella 1). Inoltre, esistono diverse situazioni cliniche (tabella 2) che sono frequentemente associate con un aumentato rischio di

tromboembolismo venoso, in quanto caratterizzate da stasi venosa e danno endoteliale.

Queste condizioni patogenetiche non si escludono reciprocamente, ma spesso interagiscono nel determinare la ipercoagulabilità e successivamente la trombosi: i pazienti con trombofilia sono infatti particolarmente a rischio qualora siano esposti ad una condizione clinica di aumentato rischio tromboembolico (ad esempio gli interventi di chirurgia maggiore e il decorso clinico post-operatorio). La stasi venosa, determinata dall'allettamento post-operatorio, è in grado di provocare sofferenza endoteliale da ipossia e quindi di neutralizzare la fisiologica attività anticoagulante dell'endotelio e la sua capacità di mantenere un perfetto equilibrio tra forze protrombotiche ed antitrombotiche. Infatti la barriera endoteliale, se integra, impedisce l'attivazione piastrinica e l'interazione tra piastrine e matrice subendoteliale; essa è in grado di produrre fisiologicamente ossido nitrico e prostaciclina, potenti inibitori dell'attività piastrinica, è in grado di ridurre la trasformazione delle proteine procoagulanti da zimogeni inattivi a forme attive, sbarrando il contatto con la superficie fosfolipidica indispensabile per l'interazione tra i fattori coagulativi, e garantisce inoltre una corretta attivazione delle proteine anticoagulanti come la proteina C.

All'eccessiva, impropria o inopportuna attivazione del sistema della coagulazione, fa seguito dapprima lo stato di ipercoagulabilità e successivamente la formazione di un trombo cui clinicamente corrisponde un episodio trombotico.

Numerosi studi recentemente pubblicati hanno confermato che nei soggetti sani c'è, in vivo, una continua modesta attivazione del sistema emostatico anche in assenza di stimoli trombogenici (16-17). Tuttavia tale attivazione non riesce ad arrivare alla soglia di manifestazione clinicamente rilevabile grazie all'attività di **feed-back** negativo esercitata dei cosiddetti anticoagulanti naturali.

3.1 Gli inibitori naturali della coagulazione

I tre principali meccanismi anticoagulanti naturali in vivo sono:

1) il sistema glicosaminoglicani-antitrombina

2) il sistema Proteina C-trombomodulina-proteina S;

3) sistema di inibizione del fattore tissutale.

- *Sistema glicosaminoglicani-antitrombina.*

L'antitrombina III (AT III) è un inibitore delle serin-proteasi con maggiore affinità per la trombina. E' sintetizzata negli epatociti, ha un P.M. di 58 KD ed è presente nel sangue ad una concentrazione di 140 microg/dl. Oltre alla trombina è in grado di inibire altre serin-proteasi del sistema emostatico, quali i fattori XIIa, XIa, IXa e Xa e sembra capace di inibire il complesso fattore VIIa-TF (18-25).

Il legame tra AT III e trombina è accelerato circa 1.000 volte dalla presenza di eparina e/o sostanze eparinosimili prodotte in vivo dai mastociti dell'apparato gastroenterico e polmonare e presenti a livello della parete vascolare endoteliale. L'eparina funge da catalizzatore anche per l'attività di altre serin-proteasi inibite dalla AT III, soprattutto del fattore IXa. Analogo effetto ha l'eparan solfato, un proteoglicano prodotto dalle cellule endoteliali.

- *Il sistema Proteina C-trombomodulina-Proteina S.*

La Proteina C è una glicoproteina con P.M. di 69 kD, è vitamina K dipendente, viene prodotta dagli epatociti ed è presente nel plasma ad una concentrazione di 4 microg/dl (26-28). Circola nel sangue sotto forma di zimogeno inattivo. Ha un'azione anticoagulante solo nella sua forma attivata aPC (proteina C attivata): agisce inattivando i fattori Va e VIIIa e quindi in ultima analisi il fattore Xa.(29-31) L'enzima responsabile della conversione della Proteina C nella sua forma attivata è la trombina che in vivo, legandosi alla trombomodulina, presente sulla superficie endoteliale, accelera ben 10.000 volte la reazione, facilitando l'attivazione della proteina C. In sintesi la trombina prodotta dall'attivazione del sistema della coagulazione esercita

anche un'azione di controllo retrogrado su un'eccessiva attivazione del sistema attivando un anticoagulante naturale (32).

Cofattore di questa serie di reazioni è la proteina S: anch'essa è una glicoproteina, vitamina k dipendente, con P.M. 69 kD, viene prodotta dagli epatociti e dalle cellule endoteliali, è presente nel plasma ad una concentrazione di 23 microg/dl, in due forme: una quota libera attiva e una quota inattiva legata a un altro regolatore del sistema, la C4 binding protein. Anche la proteina S è in grado, legandosi con la proteina C attivata, di accelerare il clivaggio del fattore V e del fattore VIII (33-35).

- *L'inibitore della via del Fattore Tissutale (TFPI).*

Un sistema anticoagulante naturale con un potenziale ruolo nella regolazione della trombogenesi in vivo è il TFPI (tissue factor pathway inhibitor=il sistema di inibizione del Fattore Tissutale).

Il TFPI è una proteina composta da 276 aminoacidi, sintetizzata dalle cellule endoteliali e dai megacariociti. Ha un P.M. di 40 KD ed è presente nel plasma sotto quattro forme ad una concentrazione totale di 100ng/ml (36-38).

1. il 75% è legato alla superficie endoteliale ed è rilasciato in circolo in seguito all'infusione di eparina (frazione "heparin releasable");
2. il 20% circola legato alle proteine plasmatiche;
3. circa il 3% circola in forma libera;
4. il restante 2 % è contenuto nelle piastrine e rilasciato in seguito a stimolazione da parte di agonisti come la trombina.

Il TFPI inibisce sia l'interazione tra Fattore VII e TF sia l'attivazione del fattore X da parte del prodotto di questa interazione: pertanto il TFPI esercita una funzione cruciale nel mantenere l'endotelio vascolare in uno stato di resistenza alla trombosi (39).

A differenza degli altri anticoagulanti naturali, non è stato finora descritto uno stato di carenza ereditaria di TFPI associato con un aumentato rischio tromboembolico.

3.2 Carenze degli anticoagulanti naturali e trombosi.

- *Carenza di antitrombina III.*

La potente azione anticoagulante dell'AT III giustifica l'incidenza di trombosi del sistema venoso nei soggetti con carenza congenita o acquisita di AT III, descritta per la prima volta nel 1970: questo difetto è trasmesso con carattere autosomico dominante e nella forma eterozigote ha una prevalenza variabile dall'1 al 3% tra i soggetti con trombosi venose idiopatiche e dello 0.3% nella popolazione generale. Nel difetto di tipo I (deficit antigenico e funzionale) si stima che approssimativamente la metà dei membri di una famiglia affetta vada incontro ad un episodio tromboembolico venoso prima dei 25 anni (40-41).

Esistono anche forme acquisite di deficit di AT III, secondarie a sindrome nefrosica (42), a grave insufficienza epatica, a coagulazione intravascolare disseminata (DIC) (43) o ad uso di estroprogestinici (44-45).

- *Carenza di proteina C e proteina S.*

I deficit congeniti di proteina C e proteina S, scoperti ripettivamente nel 1981 e nel 1984, sono trasmessi con modalità autosomica dominante e possono essere quantitativi e qualitativi (tipo I) oppure solo quantitativi (tipo II).

Si stima che la carenza di proteina C si aggiri sullo 0.5 % nella popolazione generale e dal 3 al 5% in coorti di pazienti con trombosi. I soggetti eterozigoti hanno una probabilità del 50% di aver un evento tromboembolico venoso entro i 45 anni, con un rischio relativo per un primo episodio stimato tra 8 e 11 (46-48).

La carenza di proteina S in coorti di pazienti con trombosi si aggira intorno al 2% circa, ma non ne conosciamo la prevalenza nella popolazione generale.

Il rischio relativo per episodi tromboembolici venosi è stimato essere approssimativamente di 10 (49-50).

Esistono anche carenze degli anticoagulanti naturali di tipo acquisito: deficit di proteina C di grado variabile si associano a epatopatia con grave difetto di

sintesi, a sindrome da coagulazione intravascolare disseminata (DIC), a sindrome da distress respiratorio dell'adulto o a stato postoperatorio (51-53). Deficit di proteina S può essere riscontrato in pazienti in terapia estroprogestinica (pillola anticoncezionale), gravidanza, o DIC (54-55) Stati di carenza acquisita di Proteina S possono essere correlati ad aumentati livelli plasmatici di C4 binding protein, una proteina della fase acuta dell'infiammazione (56).

3.3 Gli stati trombofilici congeniti.

- *La Resistenza alla proteina C attivata e il fattore V Leiden.*

Nell'ambito degli studi sulle alterazioni del sistema emostatico correlate alla trombofilia la scoperta più significativa è stata l'identificazione di un difetto nella risposta anticoagulante della proteina C attivata (Resistenza alla proteina C attivata: APC-R), descritto per la prima volta da Dahlback nel 1993 (57-58). La proteina C è una serin-proteasi vitamina K dipendente attivata dal complesso trombina-trombomodulina sulla superficie delle cellule endoteliali. Una volta attivato, l'enzima degrada i fattori Va e VIIIa, esplicando così la sua attività anticoagulante. L'APC-R è la causa di circa il 21 % delle trombosi venose profonde nei soggetti con età inferiore ai 70 aa, e di circa il 50 % delle trombosi venose familiari.

Il difetto molecolare responsabile di oltre il 95% dei casi di APC-R (59), è una mutazione puntiforme in posizione 1691 dell'esone 10 del gene che codifica per il fattore V che determina la sostituzione di una arginina con una glutammina nel sito di clivaggio del fattore V ad opera della proteina C attivata. Il fattore V così alterato (fattore V mutante o Leiden) non può essere degradato dalla proteina C attivata stessa, per cui conserva la sua attività procoagulante e determina uno stato di ipercoagulabilità.

Il Fattore V mutante (fattore V Arg506Gln) è la più comune tra le cause, congenite o acquisite, di trombofilia nella popolazione generale, dove si calcola abbia una prevalenza variabile a seconda delle popolazioni dal 2 al 5 % (60). Esso è associato ad un aumentato rischio trombotico ed è stato trovato in circa il 50% delle famiglie selezionate con trombofilia e in circa il

20% dei pazienti consecutivi con trombosi. Nella sua forma eterozigote aumenta il rischio dei portatori di sviluppare una trombosi di circa 7 volte. Il rischio di trombosi nei soggetti omozigoti è invece aumentato di circa 80 volte e la maggior parte dei portatori sviluppa almeno un episodio di trombosi durante la propria vita. L'espressione del fenotipo è altamente variabile e in genere le manifestazioni cliniche non sono così gravi come nei deficit degli anticoagulanti naturali (proteina C, proteina S ed antitrombina III) (61). Tuttavia, è chiaro che la presenza di fattori di rischio ambientali come un intervento chirugico, la gravidanza e soprattutto la terapia estroprogestinica aumentano il rischio di sviluppare una trombosi nei pazienti con APC-R (62).

Controverso è invece a tutt'oggi il ruolo del fattore V mutante nella patogenesi delle trombosi arteriose (63). Alcuni studi recentemente pubblicati hanno dimostrato che non c'è un incremento significativo delle trombosi arteriose in questi pazienti. Al contrario, un recente studio sui pazienti eterozigoti ha documentato un aumento del rischio di eventi ischemici cerebrali in questi soggetti (64) Inoltre, il fattore V Leiden è considerato un fattore di rischio per stroke nei bambini, negli adolescenti e persino nei soggetti con emofilia.

- *Polimorfismo genetico della Protrombina A20210G.*

Un'altra causa di ipercoagulabilità secondaria è la mutazione del gene che codifica per la protrombina (fattore II), caratterizzata dalla sostituzione di una guanina con un'adenosina nella posizione 20210 della regione 3' del gene che codifica per la protrombina, scoperta nel 1996 dallo stesso gruppo olandese, che aveva identificato nel fattore V mutato la causa della resistenza alla proteina C attivata (65-66). Tale polimorfismo è stato trovato nel 18% dei pazienti selezionati con una storia personale o familiare per trombosi venose, nel 6.2 % dei pazienti con un primo episodio di trombosi venosa profonda non selezionati e nel 2.3 % della popolazione caucasica sana.

Confrontati con i soggetti non affetti, i soggetti eterozigoti per la mutazione hanno un rischio di trombosi circa 3 volte maggiore.

E' da notare che la sostituzione nucleotidica non comporta alcuna sostituzione aminoacidica nella molecola della protrombina, perchè si trova nella zona non codificante del gene. Il meccanismo responsabile è stato chiarito solo in parte, in quanto tale polimorfismo genetico si associa ad un'aumentata attività del promotore che frequentemente determina livelli plasmatici elevati di protrombina, cha a loro volta potrebbero aumentare i livelli di trombina.

Anche la mutazione della protrombina ha una prevalenza nella popolazione generale assai alta e tipica di un poliformismo (0.3-4%) con un gradiente di distribuzione geografica che appare inverso a quello del fattore V mutato (più frequente nel Sud Europa che nel Nord).

3.4 Iperomocisteinemia e patologia trombotica.

- *Iperomocisteinemia.*

L'omocisteina è un aminoacido solforato presente nel plasma dell'individuo normale in concentrazioni variabili fra 5 e 15□ □mol/L. Prodotto della metionina attraverso la rimozione di un gruppo metilico, può essere metabolizzato a cisteina mediante un processo di transulfurazione o di nuovo a metionina attraverso un processo di rimetilazione. In queste trasformazioni sono coinvolti tre enzimi: la metilentetrafolatoreduttasi (MTHFR), enzima chiave nel ciclo dell'acido folico; la metionina sintetasi, il cui coenzima è la vitamina B12; la cistationina-□-sintetasi (CBS), che utilizza come cofattore enzimatico la vitamina B6. La carenza o anormalità funzionale di questi enzimi e/o la carenza dei loro cofattori vitaminici determinano un difettoso metabolismo dell'aminoacido e quindi il suo accumulo nel plasma in elevate concentrazioni (67).

Partendo dall'osservazione che l'iperomocisteinemia grave (omocistinuria), determinata da carenze omozigoti di cistationina-□-sintetasi e della reduttasi, è associata con eventi tromboembolici arterovenosi,

l'ipermocisteinemia moderata è stata prima ipotizzata e poi dimostrata essere fattore di rischio per trombosi arteriose e venose.

La prevalenza dell'iperomocisteinemia moderata nella popolazione generale è stata stimata essere intorno al 5%, mentre la sua prevalenza nei pazienti giovani con tromboembolismo venoso sembra essere intorno al 15% (68).

Nell'iperomocisteinemia moderata l'attività enzimatica di MTHFR o di CBS può essere ridotta al 50%: la prevalenza di questi difetti enzimatici nella popolazione generale è di circa 0.4-1.5%.

Esiste inoltre la possibilità della presenza di una variante termolabile della MTHFR legata alla presenza del polimorfismo genetico C677T.

Non in tutti i soggetti portatori della mutazione si riscontrano valori moderatamente elevati di omocisteinemia: ciò fa supporre che la loro espressione fenotipica possa essere influenzata da altri fattori, per esempio i livelli sierici di acido folico.

L'iperomocisteinemia acquisita, quindi, può essere causata da deficit di cofattori essenziali per il metabolismo della metionina:

- deficit di folati
- deficit di cobalamina (vitamina B12)
- deficit di piridossina (vitamina B6)

e da

- insufficienza renale cronica
- dall'utilizzo di alcuni farmaci

Alcuni farmaci interferiscono con il metabolismo dei folati come il methotrexate e gli anticonvulsivanti, della cobalamina come l'ossido nitrico e della vitamina B6 come la teofillina: essi possono causare iperomocisteinemia moderata. Gli estrogeni, il tamoxifene, la penicillamina e l'acetilcisteina riducono invece i livelli plasmatici di omocisteina.

- *Iperomocisteinemia e trombosi.*

I meccanismi attraverso cui gli aumentati livelli plasmatici di omocisteina determinano uno stato trombofilico con manifestazioni trombotiche venose e arteriose non sono ancora ben chiari. Studi sperimentali *in vivo* hanno

evidenzato una eccessiva attivazione dei meccanismi procoagulanti della cellula endoteliale, l'inibizione del meccanismo anticoagulante della proteina C attivata e della trombomodulina, l'inibizione di meccanismi di vasodilatazione e aggregazione piastrinica come quelli della prostaciclina e del nitrossido (68).

Parte 2 – Linee di ricerca.

1. Poliabortività e alterazioni dell'emostasi.

La poliabortività si può identificare come una delle principali cause di infertilità di coppia. Uno studio del 1999 (69) ha identificato la trombofilia congenita e/o acquisita in almeno il 50% delle donne affette da poliabortività.

La trombofilia sembra inoltre poter influenzare non solo la sterilità di coppia, ma anche in caso di gravidanza ottenuta, l'outcome della gravidanza stessa, in particolare se riferita a complicazioni quali l'insufficienza placentare, la preeclampsia, il ritardo di crescita intrauterino, abruptio placentae, morte fetale inspiegabile, abortività tardiva (70,71,72,73,74).

Si possono clinicamente distinguere due diversi stati di ipercoagulabilità: quello subclinico e l'ipercoagulabilità clinicamente evidente (trombosi).

La metodologia diagnostica della trombofilia non può prescindere dalla medicina di laboratorio che mette a disposizione del clinico test specifici e volti a identificare marcatori di trombofilia genetici e/o acquisiti (73-75-76-77).

Gli stati di ipercoagulabilità associati alle patologie trombotiche sono dimostrabili frequentemente grazie a specifici test di laboratorio di tipo quantitativo come il d-dimero, il fibrinopeptide A, il frammento protrombinico 1+2. Comunemente in pratica clinica il test più utilizzato è il d-dimero per la sua semplicità di esecuzione, per la varietà dei test qualitativi e quantitativi disponibili e per un discreto vantaggio costo\benefici.

Il d-dimero rappresenta dal punto di vista patogenetico uno dei prodotti di degradazione della fibrina non stabilizzata e la sua presenza in dosi elevate nel plasma dei soggetti analizzati identifica quindi una eccessiva produzione di fibrina. Nella comune pratica clinica l'analisi quantitativa del d-dimero rappresenta un test sensibile di facile esecuzione dal punto di vista tecnico e può semplificare la possibile identificazione dei soggetti con ipercoagulabilità e quindi anche dei soggetti portatori di trombofilia ereditaria e/o acquisita.

Nell'uso comune si è soliti ricercare gli elevati livelli di d-dimero nei pazienti con elevata probabilità pre-test strumentale di trombosi venosa profonda, ma alcuni autori hanno anche evidenziato elevati livelli di d-dimero e/o di altri marcatori di ipercoagulabilità in soggetti portatori di trombofilia allo stato asintomatico.

Alcuni polimorfismi genetici, quali la mutazione Leiden del fattore coagulativo V e la mutazione A20210G della protrombina, esercitano già un ruolo preminente tra i polimorfismi genetici ricercati in caso di poliabortività (78,79,80). I polimorfismi genetici non ancora testati in studi clinici controllati includono il □-fibrinogeno, la mutazione 1299 del fattore V, la mutazione 34 val/leu del fattore coagulativo XIII e la mutazione 4G/5G del PAI-l (74). Altre condizioni geneticamente correlate alla ipercoagulabilità, che possono essere associate a poliabortività, sono le carenze di inibitori fisiologici dell'emostasi quali proteina C, proteina S, antitrombina III (AT III) (81).

Le forme di trombofilia acquisite includono invece la presenza di una sindrome da anticorpi antifosfolipidi primitiva o secondaria, elevati livelli circolanti di fattore coagulativo VIII, forme acquisite di carenza di proteina C coagulativa e proteina S (72,73), la carenza di attività, congenita o acquisita, di fattore coagulativo XII (82,83,84,85,86,87).

Dal punto di vista classificativo possiamo distinguere la sindrome da anticorpi antifosfolipidi in primitiva o secondaria, nel caso in cui sia associata a patologie disimmuni (patologie autoimmunitarie come LES o vasculiti o patologie neoplastiche). Le manifestazioni cliniche principali sono eventi trombotici arteriosi e\o venosi e patologia della gravidanza nelle donne, in particolare la poliabortività, associate ovviamente a presenza di anticorpi antifosfolipidi. Tra le diverse varietà di anticorpi antifosfolipidi sono più comunemente associate alla patologie della gravidanza gli anticorpi anticardiolipina (classi IgM e\o IgG) o lupus anticoagulant. Tuttavia anche altre categorie di anticorpi antifosfolipidi sono state identificate e diversamente associati alle patologie trombotiche come anticorpi antifosfatidilserina, antifosfatidilcolina, antifosfatidilinositolo, anti β2-

glicoproteina I (β2-GP I); inoltre diversi studi hanno segnalato la possibile presenza di anticorpi diretti contro epitopi presenti sui fattori della coagulazione determinando tra l'altro non solo alterazioni dei comuni test di screening coagulativi come PT INR e aPTT ma talvolta anche i dosaggi quantitativi dei fattori specifici. In tale campo Braulke e coll. hanno identificato per la prima volta un'associazione tra poliabortività e deficit acquisito di fattore XII (82), ma successivamente Jones e coll. hanno riscontrato in diversi studi l'associazione di anticorpi anti-fattore XII, sindrome da anticorpi antifosfolipidi e poliabortività (83,84,85).

Inoltre, nei disordini congeniti e/o acquisiti dell'emostasi va inclusa l'iperomocisteinemia (88,89) che può avere ugualmente cause congenite e/o acquisite. L'iperomocisteinemia, infatti, è stata associata, non solo a stati di ipercoagulabilità, ma anche a un'aumentata incidenza di trombosi venose e arteriose. Tra le cause acquisite di iperomocisteinemia riconosciamo una dieta povera di folati, condizioni patologiche (ipotiroidismo, alterazioni del ricambio idrosalino, ridotta attività fisica, condizioni iatrogene-farmacologiche); le condizioni congenite che si associano a iperomocisteinemia sono invece collegate alla presenza della forma termolabile dell'enzima MTHFR (metilentetraidrofolato reduttasi) sia nella variante C677T (già studiata in altre condizioni cliniche di trombofilia) che nel polimorfismo genetico della $A_{1298}C$.

La difficile gestione della gravidanza nelle donne trombofiliche è negli ultimi anni oggetto di ricerche non solo epidemiologiche e laboratoristiche ma anche di tipo terapeutico. Non ci sono infatti consensi unanimi sulla gestione terapeutica della gravidanza delle donne trombofiliche, poiché la maggior parte degli studi disponibili in Letteratura è orientata nella prevenzione degli eventi tromboembolici della donna trombofilica in gravidanza e non verso la prevenzione della patologia della gravidanza stessa. L'utilizzo di farmaci anticoagulanti, quali le eparine a basso peso molecolare, per la prevenzione non solo degli eventi tromboembolici maggiori ma anche della patologia della gravidanza in donne trombofiliche è al

momento estremamente limitato ed un unico studio di tipo randomizzato controllato è attualmente disponibile in letteratura (90).

2. Sterilità femminile e alterazioni dell'emostasi.

Diverse sono le associazioni patologiche che comportano sterilità femminile, come precedentemente esposto, nel 5% dei casi tuttavia una chiara concausa patologica non è identificabile. Dati presenti in Letteratura sulla associazione tra sterilità femminile e ipercoagulabilità e\o trombofilia genetica\acquisita\combinata sono scarsi. Una nostra recente pubblicazione ha tuttavia sottolineato la possibile presenza di uno stato di ipercoagulabilità asintomatico presente anche in pazienti affette da sterilità femminile, legato nella maggior parte dei casi alla presenza di trombofilia molecolare per cause genetiche e\o acquisite e\o combinate (91).

Altri Autori in Letteratura hanno invece sottolineato una possibile associazione tra sterilità femminile e trombofilia per una maggiore incidenza delle varianti geniche del fattore V Leiden, della protrombina A20210G e della MTHFR C677T in pz con ripetuti insuccessi a tecniche di riproduzione assistita secondo ICSI\FIVET\IVF (92,93); tali aspetti sono tuttavia ancora da confermare su studi effettuati su vasta scala poiché i risultati presenti nelle pubblicazioni citate appaiono talvolta discordanti.

3. Scopo della ricerca.

Nel quadro delle problematiche inerenti le cause della poliabortività ricorrente e della sterilità femminile, obiettivo di questo lavoro è quello di ricercare il ruolo delle alterazioni congenite, acquisite e\o combinate dell'emostasi con tendenza alla trombofilia e degli stati di ipercoagulabilità nelle alterazioni dei processi riproduttivi. Un ulteriore approfondimento è inoltre rivolto all'associazione tra trattamenti ormonali e trombosi atipiche in donne giovani e al potenziale ruolo dei trattamenti antitrombotici nelle donne affette da abortività ripetuta e da sterilità idiopatica.

Parte 3 – Pazienti e metodi.

1. Selezione delle pazienti.

Sono state selezionate 99 pazienti di età inferiore ai 40 anni e body mass index (BMI kg/m^2) < 26, afferenti all'ambulatorio di sterilità ed infertilità di coppia del Dipartimento Universitario di Scienze Ostetriche Ginecologiche e Medicina della Riproduzione dell'Università degli Studi di Napoli "Federico II" per

- Poliabortività, diagnosticata secondo i seguenti criteri:
  - ≥2 aborti nel I trimestre di gestazione
  - ≥2 aborti nel II trimestre di gestazione
  - almeno 1 morte endouterina fetale (MEF) nel III trimestre di gestazione

- Sterilità primaria

Sono state escluse pazienti affette da concomitanti patologie:

- Malformazioni uterine, fibromi intracavitari, idrosalpinge.
- Anovulazione, insufficienza della fase luteale.
- Patologie infettive genito-urinarie in atto (Chlamydia Trachomatis, Mycoplasma pneumoniae, Ureaplasma urealyticum, Neisseria gonorreae, Trichomonas vaginalis.
- Malattie del sistema endocrino in cattivo compenso metabolico nonostante terapia farmacologia (ipotiroidismo, diabete mellito tipo I e II).
- Iperprolattinemia.
- Anomalie cromosomiche.
- Patologie infiammatorie croniche di pertinenza immunopatologica (artrite reumatoide, LES, sclerosi sistemica, vasculiti ipocomplementemiche).

Tutte le pazienti in studio sono state sottoposte ai seguenti esami diagnostici:

- β-hCG per escludere una gravidanza in corso.

- Ecografia pelvica transaddominale e transvaginale.

- Isteroscopia diagnostica.

- Monitoraggio ecografico dell'ovulazione associato a dosaggio ormonale di estrogeni (durante il periodo periovulatorio) e di progesterone (nella fase medio luteale).

- Tampone vaginale e cervicale associato a diagnosi sierologica di infezione da Chlamydia Trachomatis.

- Dosaggio ormonale di TSH, FT_3, FT_4, anticorpi antitireoglobulina, antitireoperossidasi, Cariotipo da metafasi linfocitarie.

- Dosaggio di VES, PCR (proteina C reattiva), reumatest, anticorpi antinucleo (ANA), anticorpi antinucleo estraibile (ENA), C_3, C_4

Dopo una valutazione accurata del suddetto screening sono state incluse nello studio 60 pazienti di cui:

✓ 40 affette da abortività ripetuta

✓ 20 affette da sterilità di coppia primaria idiopatica

2. Metodi.

Allo scopo di identificare alterazioni dell'emostasi nelle 60 pazienti reclutate nello studio sono stati dosati i livelli ematici di:

- Omocisteina

e i livelli plasmatici di

- D-dimero, effettuato con metodica immunochimica [Enzygnost, Bhering, Scoppito (AQ), Italy]
- Proteina C
- Proteina S
- Antitrombina III (AT III)
- Anticorpi antifosfolipidi*
  - Anticorpi anticardiolipina IgM ed IgG*
  - La presenza dell'inibitore tipo Lupus anticoagulant*

*La diagnosi di sindrome da anticorpi antifosfolidi è stata effettuata in accordo ai criteri dell'American Rheumatology Association (94,95): presenza di almeno un criterio clinico e un criterio laboratoristico.

Criteri clinici:

- trombosi arteriose/venose di qualsiasi sede, organo o parenchima
- patologia della gravidanza (aborti ricorrenti nel II trimestre di gestazione, nascite premature prima della XXXIV settimana, tre o più aborti spontanei del I trimestre non altrimenti spiegabili con anomalie geniche e/o cromosomiche)

Criteri di laboratorio:

- anticorpi anticardiolipina IgG/IgM con livelli medio-elevati (> 30 UPL) riscontrati in due o più occasioni
- presenza del lupus anticoagulant (LAC) identificato secondo le linee guida dell'International Society of Thrombosis and Haemostasis (ISTH) (24):

- prolungamento dell'aPTT al caolino, in presenza del veleno di vipera Russell, alla diluizione della protrombina, al textarin time
- correzione dell'aPTT dopo aggiunta di fosfolipidi
- esclusione di altre coagulopatie (inibitore del fattore coagulativo VIII, presenza di aumentati livelli di glicoproteina ricca di istidina)
- mancata correzione dell'aPTT dopo aggiunta di miscela di plasma povero in piastrine

Tali dosaggi sono stati effettuati con le comuni tecniche dei laboratori di analisi

E' stata inoltre ricercata l'eventuale presenza di polimorfismi genetici condizionanti la trombofilia in collaborazione con il CEINGE S.c.a.r.l. Biotecnologie Avanzate con le seguenti procedure.
Un campione di sangue (5 mL) è stato ottenuto tramite prelievo venoso e immesso in una provetta contenente EDTA come anticoagulante. Il DNA è stato estratto usando enzimi di restrizione ("Nucleon BACC2" kit, Amersham, Germany). Le pazienti dei due gruppi sono state testate per i seguenti polimorfismi genetici utilizzando la metodica PCR (reazione polimerasica a catena) per l'amplificazione genica con specifici primers e l'apparecchio Light Cycler (Roche, Milan, Italy).
Polimorfismi genetici ricercati.

- Polimorfismo genetico C677T della MTHFR
- Polimorfismo genetico 1691 del fattore coagulativo V (fattore V Leiden, FVL)
- Polimorfismo genetico A20210G della protrombina (PTHRA20210G)

3. Gruppo controllo.

Nel gruppo controllo costituito da soggetti volontari comparabili per età, sesso, etnia e caratteristiche antropometriche sono state reclutate 30 pazienti. Tutti i soggetti selezionati presentavano anamnesi positiva per una o più gravidanze spontanee e condotte a termine senza complicazioni (i.e. insufficienza placentare, preeclampsia, gestosi EPH, ritardo di crescita intrauterino, abruptio placentae). Nei soggetti selezionati non era presente alcun rilievo anamnestico personale e/o familiare (parenti di I grado) di patologie trombotiche giovanili né del versante venoso (trombosi venosa profonda, trombosi venosa superficiale, tromboembolia polmonare) né di quello arterioso (stroke, sindromi coronariche acute, arteriopatia ostruttiva degli arti inferiori).

Sono state escluse inoltre dal gruppo controllo donne con anamnesi positiva per patologie ostetriche quali aborti del primo trimestre, morte intrauterina (MEF).

Allo scopo di identificare alterazioni dell'emostasi in tali soggetti sono state ricercate le cause di trombofilia geneticamente determinata indicate già per il gruppo di studio.

Parte 4 – Risultati.

1. P. Di Micco, **M D'Uva**, M. Romano, B. Di Micco, A. Niglio. Stroke due to left carotid thrombosis in moderate ovarian hyperstimulation sindrome. Thromb Haemost 2003; 90: 957-60 [Case Report]

Le associazioni tra terapie ormonali e trombosi arteriose e\o venose (i.e. contraccezione o terapia ormonale sostitutiva) sono ben note; pochissimi dati, peraltro non univoci, sono invece disponibili in Letteratura per le associazioni tra iperstimolazione ovarica controllata (COH) e/o sindrome da iperstimolazione ovarica (OHSS) e eventi trombotici. Brevi serie di case reports sono disponibili per le trombosi venose anche di distretti atipici come il distretto venoso profondo degli arti superiori. Ancor più rara è l'associazione tra COH o OHSS con le patologie trombotiche arteriose in particolare lo stroke ischemico. Sono disponibili in Letteratura meno di 20 casi in cui tale associazione clinica si è verificata. Riportiamo la nostra esperienza clinica nella gestione di un caso di stroke ischemico a causa di una trombosi della carotide interna sn su arteria sana in una giovane donna sottoposta ad un protocollo di iperstimolazione ovarica controllata con conseguente OHSS moderata.

Case Report

Stroke due to left carotid thrombosis in moderate ovarian hyperstimulation syndrome

Introduction

Ovarian hyperstimulation syndrome (OHSS) is the most serious complication of sterility treatment, in particular of pharmacological ovulation induction therapy (i.e. controlled ovarian hyperstimulation, COH), requiring hospitalization in up to 2% of cases (1). OHSS is characterized by ovarian enlargement, abdominal distention, electrolyte imbalance, ascites, hypovolemia and haemoconcentration and is classified as mild, moderate and severe based on clinical signs, symptoms, laboratory findings and imaging (2). Thromboembolic disorders may occur in patients with OHSS and are considered to be contributed to by haemoconcentration, particularly in those subjects with severe OHSS (2). However, a number of thromboembolic accidents have been observed in moderate OHSS and this might be related to concomitant inherited or acquired alterations of haemostasis (3-4). In this respect, several conditions other than OHSS characterized by increased sexual hormones serum levels (i.e. pregnancy, hormone replacement therapy or oral contraceptives) are considered as thrombotic risk factors because of their association with thromboembolic events (5-7). Pharmacological treatment of sterility induces a sustained increase in the release of oestrogens and progesteron from ovarium due to the simultaneous maturation of multiple follicles (2, 8). Therefore, one may hypothesize that the occurrence of thromboembolic events in OHSS is related to the potential thrombophilic action exerted by oestrogens and/or progesterone (5-7). We report here about a case of ischemic stroke due to a full carotid thrombosis in a young woman who underwent pharmacological treatment of sterility and had no relevant thrombotic risk factors.

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Case history

In March 2002, a 32 year old non-smoking woman with 8 years of primary sterility, without a personal history of juvenile thrombosis and with no clinical risk factors for thrombotic events, was hospitalized because of a suspicion of OHSS subsequent to COH. During the last 5 years she had undergone 8 unsuccessful cycles of COH at 3-12 month intervals. Nine months after the last COH cycle she received a new COH treatment based on administration of urinary-follicle stimulating hormone (i.e. u-FSH, 150 UI daily for 10 days), to obtain a multiple ovarian follicles induction, followed by administration of human chorionic gonadotropin (i.e. hCG, 10.000 UI once) to induce ovulation. Plasma 17-β estradiol concentration was 3800 pg/mL on the day of ovulation induction. We do not have any data regarding the concentration of 17-β estradiol during the previous COH cycles. However, there was no difference of prescription between the present COH and the previous ones. Physical examination showed abdominal distention. Laboratory findings showed normal haemoconcentration (hematocrit 39.6%, normal range 36-44%), normal plasma sodium levels (136 mEq/L, normal range 130-155 mEq/L), normal plasma potassium levels (4.1 mEq/l, normal range 3.5-5.5 mEq/L). Ultrasound scan showed ovarian enlargement (maximal diameter of right and left ovaries was 6.5 cm and 7.2 cm, respectively) with multiple post-ovulatory follicles (i.e, six in each ovarium) and a small ascitic slice. Based on clinical, laboratory and imaging data, the patient was diagnosed a moderate OHSS according to Golan et al (2).

Forty-eight hours after hospitalisation the patient developed right sided hemiplegia with central facial nerve paresis and reduced right field vision but with intact consciousness and sensory activity. A vascular ultrasound examination of carotid arteries showed left carotid thrombosis. A subsequent angiography confirmed full thrombosis of left carotid artery as the cause of the ischemic stroke (Fig. 1). Previous head/neck trauma possibly leading to traumatic carotid dissection was ruled out based on a thorough anamnesis. Also, a cardiac ultrasound scan ruled out the possibility of cardiac thrombosis thus making it unlikely that the left carotid involvement was due to cardiac embolism. Blood pressure was 120/65 mmHg, and no abnormalities were found in total cholesterol (170 mg/dL normal value < 220 mg/dL), LDL-cholesterol (100 mg/dL, normal

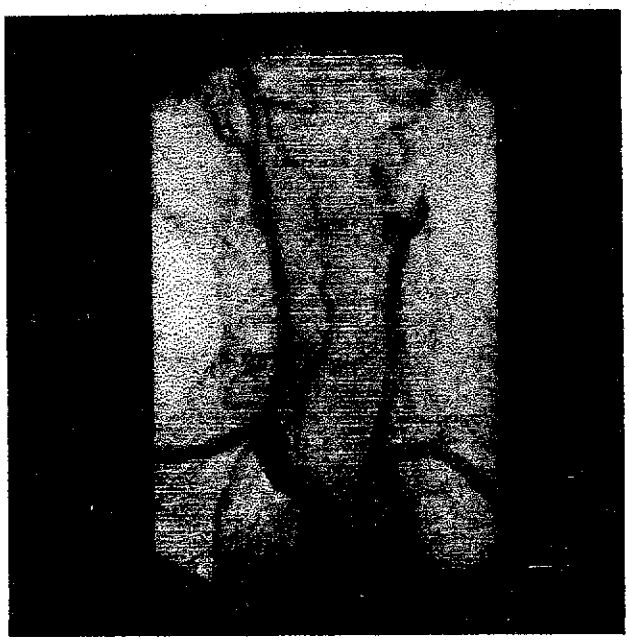

Figure 1: Left carotid thrombosis at angiography
Angiography showing the full left carotid thrombosis and the normal imaging of right carotid in the patient affected by stroke during moderate OHSS.

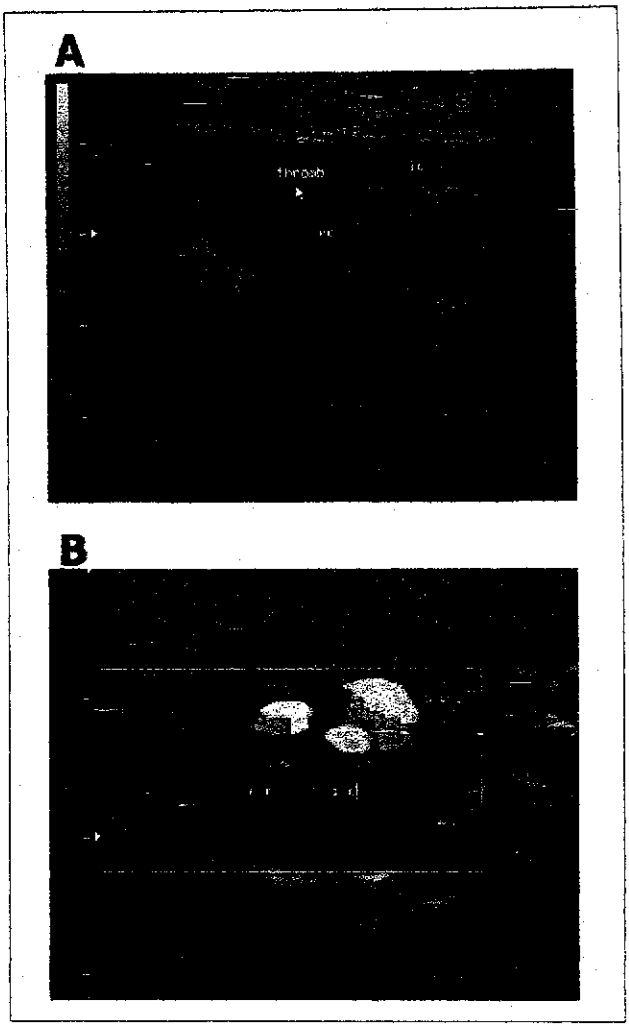

Figure 2: Left internal carotid thrombosis and its disappearance after treatment
A: longitudinal ultrasound vascular scan showing full thrombus of left internal carotid artery (arrow); B: transversal ultrasound vascular scan showing patency of left internal carotid thrombosis in the same patient after therapy. (ic, internal carotid; ec, external carotid).

value 80-130 mg/dL), HDL-cholesterol 50 mg/dL, normal value > 35 mg/dL), blood glucose (92 mg/dL, normal value 70-110 mg/dL), prothrombin time (PT measured as INR, 0.92, normal value 0.8-1.2), activated partial thromboplastin time (aPTT measured as ratio, 0.98, normal value 0.8-1.2), fibrinogen (296 mg/dL, normal value 220-400 mg/dL), platelet count ($238x10^3/mm^3$, normal value $100x10^3$-$400x10^3$), white blood cell count ($8.6x10^3/mm^3$, normal value $4x10^3$-$10x10^3/mm^3$), red blood cell count ($4.6x10^6/mm^3$, normal value $4.x10^6$-$6x10^6/mm^3$). We also measured molecular markers of thrombophilia such as D-dimer (1,000 ng/mL, normal values < 280 ng/mL), FDP (> 10 µg/mL, normal values <10 µg/mL), prothrombin fragment F1+2 (3.2 nM, normal values 0.4-1.1 nM), and clot lysis time (115 min, normal values 120-300 min).

The patient was treated with intravenous heparin injection (bolus injection of 5000 IU followed by 1000 IU/h for 24 hours, modulated on aPTT ratio performed every 6 hours) and glycerol infusion (250 cc every 12 hours). We chose heparin rather than urokinase or t-PA because of a previous report of intracranial haemorrage in a patient with OHSS-related stroke (9). Forty eight hours after therapy there was a dramatic improvement in clinical conditions. Also, there was a reduction of the hypercoagulable state as assessed by determination of aPTT ratio (2.2), D-dimer (580 ng/mL), FDP (<10 µg/mL), prothrombin fragment F1+2 (0.8 nM), and clot lysis time (65 min). Therefore, heparin was discontinued and aspirin treatment (150 mg/die) was started. Because of the improvement in clinical conditions (i.e. a change in the Barthel ADL Index from 60 on admission to 85) the patient was discharged and aspirin therapy was continued for 6 months. Vascular ultrasound imaging showed disappearance of the carotid thrombosis (Fig. 2). We investigated the existence of acquired and/or inherited thrombophilia and found normal levels of anticardiolipin antibodies (IgG 5 GPL U/mL, normal values < 11 GPL U/mL; IgM 4 MPL U/mL, normal values < 11 MPL U/mL), normal activated protein C resistance (APCr 1.14, normal values > 0.77), normal plasma levels of homocysteine (8.2 µmol/L, normal values 5-15 µmol/L), plasminogen activator inhibitor type 1 (PAI-1, 31

ng/dL, normal values 4-45 ng/dL), clotting factor VIII (125 %, normal values 85-150%), and absence of lupus anticoagulant inhibitor. Moreover, we did not find any alteration in plasma levels of clotting inhibitors (i.e. C Protein 104% of activity, normal values 70-120%; S Protein 102 % of activity, normal values 70-120%; S Protein antigen 105%, normal values 65-125%; AT III 99 % of activity, normal values 80-120 %), nor was any mutation in prothrombin gene and clotting factor V gene detected, the patient being normal homozygous for $PTHRA_{20210}G$ and FVL. However, we found heterozygosity for $C_{677}T$ mutation of methylenetetrahydrofolate reductase (i.e. MTHFR $C_{677}T$).

Discussion

OHSS is a potentially serious complication during sterility treatment, in particular in patients undergoing COH. An increased capillary permeability leading to enhanced fluid extravasation seems to play a major pathogenic role (8, 10). Also, an imbalance in the renin-angiotensin system and an increased plasma concentration of free vascular endothelial growth factor are involved (8, 10). Thromboembolic events may occur in patients with OHSS (1-2). The mechanism whereby OHSS induces thrombotic disorders is uncertain. It has been suggested that beside haemoconcentration (2), increased plasma levels of fibrinogen and tissue factor together with decreased AT III and tissue factor pathway inhibitor levels may play a role (3-11). Increased levels of oestrogens induced by pharmacological ovulation induction also may contribute to the OHSS-hypercoagulable state due to their known thrombophilic effects (5-7). This might explain the occurrence of thromboembolic events in patients with OHSS (9, 12-15). The interest of our case mainly relies in the thrombotic involvement of a large arterial vessel and in particular of the left internal carotid artery in a patient with OHSS, in the absence of any thrombotic risk factor. Thrombosis of upper extremities, both arterial or venous, is very rare compared with that involving lower extremities (16). Moreover, our patient had normal blood pressure, normal total cholesterol, HDL-cholesterol, normal LDL-choelsterol, normal body mass index. She did not have diabetes nor did she have any laboratory marker of inherited or acquired thrombophilia (i.e. normal levels of anticoagulant proteins, no mutations of clotting factor II and V inducing inherited thrombophilia, absence of lupus anticoagulant inhibitor, normal levels of anticardiolipin antibodies, as well as of PAI-1, APCr, homocysteine, and clotting factor VIII) (17). However, our patient was heterozygous for $C_{677}T$ mutation of MTHFR. MTHFR homozygous subjects are known to be at risk of thrombosis (18-19), even though this is controversial (20). Whether $C_{677}T$ mutation of MTHFR was at least in part responsible for the ischemic stroke in our patient is difficult to establish.

We hypothesize that ovarian stimulation leading to increased release of oestrogens might have contributed to the occurrence of this thrombotic event due to the known thrombophilic action of oestrogens. In partial support of this hypothesis 17-β estradiol plasma levels during ovarian stimulations were 20 fold higher than normal levels. However, a strict relationship between oestrogens and thrombophilia in this particular case is difficult to establish because we do not have any follow up data on plasma estradiol during the course of the disease.

This report suggests that patients candidate to pharmacologic treatment of sterility such as ovarian gonadotropin stimulation for ovulation induction should undergo a thorough evaluation in order to rule out thrombophilia. This should include assessment of $C_{677}T$ MTHFR mutation and other markers of inherited thrombophilia. Also, this study raises the question as to whether patients who are potentially at risk for thrombotic events should be given pharmacologic treatment for sterility and, if so, whether a thromboprophylaxis might decrease their thrombotic risk.

Pierpaolo Di Micco, Maristella D'Uva, Marco Romano, Biagio Di Micco, Alferio Niglio

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Erratum

In the article by Look, et al. entitled "Pooled analysis of prognostic impact of uPA and PAI-1 in breast cancer patients" published in *Thrombosis and Haemostasis* September 2003 (Thromb Haemost 2003; 90: 538-48) the name of the author P. O. Bendahl and his affiliation the Department of Oncology, University Hospital, Lund, Sweden were published incorrectly.

2. P Di Micco, **M D'Uva**, G De Placido et al. The role of d-dimer as first marker of thrombophilia in women affected by sterility: implications in pathophysiology and diagnosis of thrombophilia induced sterility. Journal of Translational Medicine 2004; 2: 38 [Original investigation]

Un'attenta selezione delle pazienti afferenti presso l'ambulatorio di sterilità ha permesso di selezionare un gruppo di esse senza apparenti cause di sterilità o poliabortività. Una prima fase della ricerca era rivolta allo screening delle altre cause etiopatologiche di sterilità femminile o poliabortività (i.e. cause anatomiche, cause genetiche, cause infettive, cause endocrinologiche, cause infiammatorie). Sono state così selezionate 39 pazienti affette da sterilità idiopatica o abortività ripetuta. Una prima valutazione laboratoristica, che prevedeva il dosaggio del d-dimero, ha permesso di dividere la popolazione selezionata in due gruppi: il gruppo A che includeva pazienti con elevati livelli di d-dimero, mentre il gruppo B includeva le pazienti con livelli di d-dimero nella norma. Il gruppo di controllo era costituito da un campione di 15 donne comparabili per caratteristiche antropometriche senza storia anamnestica di sterilità, poliabortività e\o patologie trombotiche (i.e. trombosi arteriose e\o trombosi venose) (sottogruppo C).

Successivamente al dosaggio di d-dimero tutti i soggetti facenti parte dello studio venivano ulteriormente indagati per la ricerca delle cause di trombofilia eventualmente presenti.

Venivano effettuati inoltre in tutti i soggetti in studio dosaggio della β-HCG e esame ecocolordoppler venoso degli arti inferiori per escludere eventuali altre cause di incremento del d-dimero.

I risultati hanno evidenziato una stretta correlazione tra incremento del d-dimero quale marker di ipercoagulabilità asintomatica legato alla presenza di

una trombofilia molecolare per cause genetiche, acquisite o combinate. Nel gruppo A, infatti, l'80% delle pazienti presentava aumento di d-dimero e trombofilia molecolare, mentre nel gruppo B una quota minore delle pazienti era portatrice di uno stato di trombofilia allo stato asintomatico senza associazione con elevati livelli di d-dimero (50%), mentre tale condizione era presente nel 33% dei soggetti del gruppo di controllo. Le differenze statistiche raggiungevano la significatività se venivano confrontati il gruppo A sia con il gruppo B che con il gruppo C dimostrando così che il d-dimero, se aumentato, può essere un utile markers di ipercoagulabilità asintomatica in pazienti affette da abortività ripetuta o sterilità; tale dato potrebbe risultare utile nella gestione quotidiana delle pazienti afferenti presso gli ambulatori per problemi di sterilità o abortività ripetuta in quanto di rapida esecuzione e di basso costo allo scopo di individuare precocemente pazienti potenzialmente portatrici di trombofilia molecolare per cause genetiche o acquisite o combinate.

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Research

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The role of d-dimer as first marker of thrombophilia in women affected by sterility: implications in pathophysiology and diagnosis of thrombophilia induced sterility

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Abstract

Background: D-dimer is considered a marker of hypercoagulable state and of endogenous fibrinolysis, so increased d-dimer is detectable in patients affected by thrombosis. Yet, several studies showed that also infertility, in particular secondary infertility due to recurrent fetal losses, has been often related to thrombotic events, in particular in women carrying thrombotic risk factors such as inherited thrombophilia ($MTHFR_{C677T}$, $PTHR_{A20210G}$, Factor V Leiden polimorphisms and/or inhAfter this screening we selected 39erited protein C, protein S, AT III deficiency) or acquired thrombophilia (primary antiphospholipid syndrome, acquired protein C, protein S, AT III deficiency, drugs induced thrombophilia). However, because its high predictive negative value in case of suspected thrombosis, increased d-dimer has been often associated to subclinical thrombophilia. The aim of this study is to investigate the role of d-dimer as first marker of thrombophilia in women affected by unexplained infertility and subsequently to search the cause of increased d-dimer, such as inherited and/or acquired thrombophilia.

Patients and Methods: We selected 79 patients with unexplained primary or secondary infertility. We excluded 40 patients affected by hydrosalpinx, uterine fibroids, uterine malformations, endocrinological and immunological diseases, luteal insufficiency, cytogenetical alterations. All remaining 39 patients were tested for d-dimer and divided in two groups: the patients of group A (25 patients) showed increased plasma d-dimer, in group B were included 14 patients with normal plasma level of d-dimer. After this step all 39 patients were screened for $MTHFR_{C677T}$, $PTHR_{A20210G}$, Factor V Leiden polimorphisms, protein C, protein S, AT III, anticardiolipin IgM and IgG, lupus anticoagulant. In the control group were included 15 age matched women without sterility problems referred to our outpatient's section of vascular medicine for suspected deep venous thrombosis.

Statistical analysis was based on χ^2 test, differences were considered to be significant if $p < 0.05$.

Results: D-dimer was increased in 25/39 and 20/25 showed inherited/acquired thrombophilia while patients with normal d-dimer showed inherited/acquired thrombophilia in 7/14 (p: < 0.05, s).

Discussion: D-dimer is a well known marker of hypercoagulable state, in particular its high predictive negative value in case of suspected thrombosis has been recognised by several reports. Yet, increased d-dimer has been identified also for subclinical thrombophilia besides for vascular thrombosis. Our data, in fact, for the first time suggest an interesting role of d-dimer to identify women affected by unexplained primary or secondary infertility and thrombophilia. So, probably there is a role for d-dimer in these subjects for its predictive positive value. Of course, further data on large based population are needed to confirm our results, because these findings may speed up a diagnostic screening in these patients also for a good cost/effectiveness of this test.

Introduction

D-dimer is considered a marker of hypercoagulable state besides of endogenous fibrinolysis, so increased d-dimer is detectable in patients affected by arterial and/or venous thrombosis [1]. Yet, several studies showed increased d-dimer also in patients affected by subclinical thrombophilia without ongoing thrombosis [2]. Moreover, also in other clinical conditions, such as chronic inflammation as infectious disease (also as marker of disseminated intravascular coagulation if sepsis is associated) as cancer as necrosis as eldership and pregnancy we may observe an increase of plasma d-dimer [3-8]. So, for this reason d-dimer test is usually used in clinical management for its high predictive negative value in suspected thrombosis, particularly in deep vein thrombosis (DVT) [9-11]. However, several studies showed that frequently women affected by sterility, in particular secondary sterility for recurrent foetal losses, may be affected by an underlying inherited and/or acquired thrombophilia [12-20]. Besides, common thrombotic risk factors which include also a bad lifestyle (e.g. obesity, non regard to Mediterranean diet, sedentary life), a lot of molecular thrombotic risk factors such as inherited or acquired clotting inhibitor deficiency (i.e. protein C, protein S, antithrombin III), inherited thrombophilia (factor V Leiden, prothrombin A20210G mutation), primary or secondary hyperhomcysteinemia, primary or secondary antiphospholipid syndrome and increased plasma factor VIII levels have been identified [21]. Furthermore, these molecular alterations may be also associated in some subjects so inducing gene-gene interactions and/or gene-enviromental interactions [22-24]. So, because the high incidence of clotting abnormalities in these patients, according to the data of Brenner et al. [24,25], we investigated the role of d-dimer as first marker of thrombophilia in women affected by sterility in order to identify causes of increased d-dimer and probably of the induced sterility.

Patients and Methods

We selected 79 women affected by primary or secondary sterility (due to three or more fetal losses) referred to our sterility center. We excluded 40 patients affected by hydrosalpinx, uterine fibroids, uterine malformations, luteal insufficiency, anovulation, cytogenetical alterations, infectious diseases, endocrinological diseases (ie diabetes, subpituitarism), and by immunological diseases (inherited and/or acquired immunodeficiency, rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, vasculitis).

After this screening we selected 39 patients (12 affected by primary sterility and 27 by secondary sterility due to recurrent foetal loss). These 39 patients were tested for d-dimer. D-dimer was measured by several methods [26]; d-dimer were tested randomly in various periods of the ovarian menstrual cycle in 31 patients, in one patient during menstrual bleeding and in seven patients during hormonal therapy in order to obtain controlled ovarian hyperstimulation (COH). Following d-dimer examination patients were divided in two different groups: group A including 25 patients with increased d-dimer levels and group B including 14 patients with normal d-dimer levels. As control group we selected 15 age-matched women, without sterility problem in their anamnesis, referred to our outpatient's section of vascular medicine for suspected deep venous thrombosis.

Subsequently d-dimer evaluation, in order to identify a possible inherited and/or acquired thrombophilia, all patients were screened for methilene-tetra-hydro-folate-reductase C677T gene polimorphism ($MTHFR_{C677T}$), Factor V Leiden gene polimorphism (FVL), prothrombin A20210G gene polimorphism ($PTHR_{A20210G}$), protein S deficiency, protein C deficiency, antithrombin III deficiency (AT III), lupus anticoagulant, IgM and/or IgG anticardiolipin autoantibodies [22,27]. Moreover, all patients showing increased d-dimer were tested also for β-human-corionic-gonadotropin (β-HCG) to exclude early preg-

nancy, and lower limb ultrasound vascular examination associated to compression ultrasonography (CUS) to exclude a lower limb deep venous thrombosis (DVT); both conditions, in fact, are well known conditions associated to increased d-dimer [4,9-11].

Furthermore, patients with increased d-dimer (group A, 25 patients) and patients with normal d-dimer (group B, 14 patients) were compared also for possible differences in molecular markers of inherited and/or acquired thrombophilia.

Statistical analysis was based on χ^2 test, differences were considered to be significant if $p < 0.05$.

Results

We found thrombophilia in group A, 80%, and in group B, 50%, so thrombophilia rate in all 39 selected was 65% if we consider together group A (i.e. women affected by sterility and showing increased d-dimer) and group B (i.e. women affected by sterility with normal d-dimer levels) (table 1, 'see additional file 1').

Twenty patients of group A (80%) affected by sterility with increased d-dimer levels, showed inherited and/or acquired thrombophilia [(six $MTHFR_{C677T}$ homozygosity, four FVL heterozygosity, five $PTHR_{A20210G}$ heterozygosity, three inherited Protein S deficiency, two showing combined defects (one $MTHFR_{C677T}$ homozygosity associated to protein S deficiency and one $MTHFR_{C677T}$ homozygosity associated to FVL heterozygosity), none protein C deficiency or AT III deficiency, none positive for the presence of lupus anticoagulant, none with increased anticardiolipin autoantibodies IgM and/or increased anticardiolipin autoantibodies IgG)] (table 2, 'see additional file 2'). Remaining five women of the group A did not show molecular thrombophilia, but in their anamnesis we found some possible correlation with an acquired thrombophilia: controlled ovarian hyperstimulation in one patient, monthlies in one patient, early pregnancy in one patient, miscarriage in one patient, none apparent cause in 1 patient; among them two of these five patients were heterozygous for $MTHFR_{C677T}$. Furthermore, two patients of group A carrying inherited thrombophilia for the presence of heterozygous FVL and increased d-dimer revealed previous DVT with following pulmonary embolism in their anamnesis. Data of patients of group A are summarised in table 2 ('see additional file 2').

Seven patients of group B (50%) showed inherited and/or acquired thrombophilia (one $MTHFR_{C677T}$ homozygosity, one FVL heterozygosity, five $PTHR_{A20210G}$ heterozygosity, none inherited Protein S deficiency, protein C deficiency, AT III deficiency, none presence of lupus anticoagulant, none with increased anticardiolipin autoantibodies IgM and/or IgG), as reported in table 2 ('see additional file 2'). All remaining seven patients of group B showed all heterozygosity for $MTHFR_{C677T}$. Moreover, none patients of group B revealed previous DVT and/or pulmonary embolism.

Five patients of group C (i.e. control group) (33.3%) showed increased d-dimer as molecular markers of ongoing proximal DVT confirmed by ultrasound vascular examination associated to CUS; moreover, all five patients revealed an underlying inherited and/or acquired thrombophilia (three $MTHFR_{C677T}$ homozygosity, one protein S deficiency, one combined thrombophilia: FVL heterozygosity associated to protein S deficiency). Data of patients of group C are summarised in table 2 ('see additional file 2').

In all groups positivity for anticardiolipin antibodies or lupus anticoagulant mimicking a primary antiphospholipid syndrome (APS) was not discovered.

So, as showed in table 3 ('see additional file 3'), increased d-dimer is frequently associated with thrombophilia in women affected by sterility, while this association is less present in patients with normal d-dimer, and this difference reaches statistical significance ($p < 0.05$); furthermore thrombophilia is more frequent in group A than in control group (i.e. group C) and also this difference reaches statistical significance ($p < 0.05$); finally, thrombophilia in group B is more frequent than in control group (i.e. group C), but this difference does not reach statistical significance (p: 0.08, ns).

Discussion

In this report for the first time the role of d-dimer was investigated in diagnostic screening of patients affected by sterility and this is a really innovative data available in this clinical setting.

D-dimer is a fibrin degradation product which usually is extensively screened in patients with suspected thrombosis and/or pulmonary embolism [9]. An increased plasma d-dimer might have a predictive positive value for DVT and/or pulmonary embolism, but because increased d-dimer has been observed also in several conditions not associated with ongoing thrombosis (malignancy, chronic inflammation, infections, acute coronary syndromes, necrosis, eldership) [3-9] the really interesting role of d-dimer in this clinical setting is for its high negative predictive value as reported by Bounameaux et al. in a series of patients with suspected pulmonary embolism [9]. However, increased d-dimer has been observed also in subjects affected by thrombophilia (i.e. inherited thrombophilia and/or acquired thrombophilia) showing

hypercoagulable state without ongoing thrombosis as reported by Arkel et al. and Humphries et al. [2,23].

So, our data showed that patients of group A, carrying increased d-dimer, has been extensively screened for inherited and/or acquired thrombophilia and 80% of them revealed a well known molecular condition associated to hypercoagulable state which may explain increased d-dimer levels (table 2, 'see additional file 2'). Moreover, this our clinical and laboratory screening reaches statistical significance compared to group B and group C (table 3, 'see additional file 3'). Furthermore, five patients with increased d-dimer did not reveal inherited and/or acquired thrombophilia, but a thorough anamnesis and a clinical evaluation permitted to identify other causes of increased d-dimer in four of these patients: one patient showed early pregnancy (confirmed by β-HCG measurements and following ultrasound scan), a known condition associated to hypercoagulability and increased d-dimer [4,28,29], one patient revealed an early abortion, confirmed by following decrease of β-HCG, and increased dimer levels might be related to uteroplacental thrombosis and/or necrosis [30], one patient was ongoing to controlled ovarian stimulation and this condition may be associated to alteration of haemostasis with a trend toward thrombophilia [31,32] and one patient showed ongoing monthlies, a condition associated to wound healing which involves also clotting factors and might explain increased d-dimer [33]; remaining one patient showed increased d-dimer for unknown causes probably related to not well studied thrombophilia [34] or idiopathic thrombophilia and/or other conditions although we excluded in our selection criteria several other diseases associated to increased d-dimer.

So for the first time we showed an interesting and relevant role of d-dimer in the screening of sterility causes, particular an underlying thrombophilia may be suspected in pathophysiology of sterility if plasma d-dimer is increased. However, also an evaluation of other conditions associated to increased d-dimer (e.g. chronic inflammation, immunopathological diseases, infectious diseases, cancer, necrosis, eldership, pregnancy, controlled ovarian stimulation, monthlies) should be performed in order to avoid a misinterpretation.

Also group B, with normal d-dimer levels, showed an increased rate of thrombophilia (50%, table 1, 'see additional file 1'), so confirming one more time the clear relationship between thrombophilia and sterility, even if these data did not reach statistical significance compared to group C (table 3, 'see additional file 3'). Yet, patients of group B, although affected by thrombophilia and sterility did not show increased d-dimer. This finding might be explained by several causes and a laboratory mistake cannot be excluded; furthermore, these patients of group B may show also transient and/or silent thrombophilia which may trigger a hypercoagulable state if associated to other causes (i.e. acquired conditions associated to thrombophilia) during their natural history and our evaluation of d-dimer might be done during a not-hypercoagulable transient state.

An extensive screening of causes of increased d-dimer in our population was also performed. The association between thrombophilia and sterility due to recurrent foetal loss is well known as reported by several reports [12-20] and also by our data. However, recently an association between primary sterility and thrombophilia has been underlined such as also between thrombophilia and repeated in vitro fertilisation failures [35,36].

A clear relationship between thrombophilia and recurrent foetal loss has been reported for inherited deficiency of clotting inhibitors (i.e. protein C deficiency, protein S deficiency, AT III deficiency) [20,36], but we did not find in our population this strong association (only four cases of protein S deficiency, one of these associated to $MTHFR_{C677T}$ homozygosity, and none case of protein C deficiency and/or AT III deficiency). However, this aspect seems to be in agreement with other reports in which other thrombophilic conditions were more frequent than clotting inhibitor deficiencies (e.g. FVL, $MTHFR_{C677T}$ homozygosity, antiphospholipid syndrome and so on) [12-20].

FVL gene polymorphism has been frequently found in women affected by recurrent fetal loss, although the frequency of FVL differs in each study [15,24]. These differences could be related, besides to ethnic background, also to different inclusion criteria of investigated patients. However, FVL is associated to sterility also in our study (four cases in group A and one case in group B; table 2, 'see additional file 2').

An increased $MTHFR_{C677T}$ homozygosity has been found in our study population (six cases in group A and one case in group B), so confirming a clear role of homocysteine metabolism and of the related hypercoagulable state in sterility pathophysiology [38-40]. Of course, MTHFR gene polymorphism and related homocysteine metabolism may influence sterility also through folic acid and vitamin B12 deficiency due to uncorrected diet and/or lifestyle [41].

We found also an increased frequency of $PTHR_{A20210G}$ in women affected by sterility (five cases in group A and five cases in group B), and these data seem to be different from data reported by Pickering et al [42] and Deitcher et al [43] and in agreement with data reported by Brenner et al

[24,25]. As we previously underlined, these differences could be related to inclusion criteria established by Investigators of each study and also to an ethnic background; this gene polymorphism, in fact, is more frequent in Southern Europe than in Northern Europe [44,45].

A really interesting data is the absence of APS from our study population and this data differs from data of the Literature. A possible explanation could be offered by different selection criteria: we exclude, in fact, women with immunopathological diseases (e.g. rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, vasculitis), so excluding the most common causes of secondary APS and so searching only primary APS that is more rare than primary [46].

In conclusion, in this investigation both groups of women affected by sterility, group A and B, showed increased incidence of thrombophilia compared to control group (group A vs group C, p: < 0.05, s; group B vs group C, p: 0.08, ns; table 3, 'see additional file 3'), so confirming, one more time the relevant role of thrombophilia in pathophysiology of sterility. So, the first relevant data we offer in this study is the role of d-dimer in the screening of sterility causes in order to early suspect an underlying thrombophilia; this screening, as also showed by our data, is in agreement with an elevated frequency of thrombophilia in women affected by sterility (80 % in group A, 50% in group B, 65% if we consider together group A and B). Of course, although several Authors already reported the association between thrombophilia and recurrent foetal loss we may testify that probably the role of thrombophilia is an underestimated problem if we consider all sterility conditions because usually thrombophilia is screened only for repeated foetal loss and not screened in any case of unexplained sterility as in this study.

So, based on our data further studies on large population are needed not only to confirm our results but also to focus a possible different prognosis of these groups, in particular to sterility prognosis.

Conclusion

Our data demonstrated a clear role of thrombophilia in patients affected by sterility, but suggesting a clear diagnostic role of increased d-dimer in a lot of these patients. This diagnostic screening of thrombophilia in women affected by sterility, based on the d-dimer levels, may also represent a really speed method to suspect thrombophilia in these subject and has also a good cost/benefit ratio, although other causes of increased d-dimer should be always considered. In a second step, if increased d-dimer levels are present causes of hypercoagulable state may be investigated (i.e. inherited thrombophilia and/or acquired thrombophilia). This approach may play a role not only in differential diagnosis of sterility but also in the early diagnosis of sterility due to thrombophilia. After the first step in which d-dimer may be evaluated, causes of increased d-dimer should be subsequently identified in order to start a possible antithrombotic treatment soon.

Nevertheless thrombophilia may be present in few cases also in subjects with normal d-dimer, it should be investigated always if other causes of sterility are not present.

So, we strongly suggest to test d-dimer in patients affected by sterility as first step of a possible underlying thrombophilia in order to early identify the cause of thrombophilia and its prompt treatment but other data should be confirmed by further investigations on large based population.

Additional material

Additional File 1

Thrombophilia frequency in studied groups
Click here for file
[http://www.biomedcentral.com/content/supplementary/1479-5876-2-38-S1.doc]

Additional File 2

Thrombophilia in studied groups
Click here for file
[http://www.biomedcentral.com/content/supplementary/1479-5876-2-38-S2.doc]

Additional File 3

statistical analysis according with χ^2 method
Click here for file
[http://www.biomedcentral.com/content/supplementary/1479-5876-2-38-S3.doc]

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3. P Di Micco, **M D'Uva**. The role of low molecular weight heparin to prevent miscarriage in thrombophilic women. Thromb Haemost 2005; 94: 897-898 [Editorial Comment]

L'associazione tra trombofilia e abortività ripetuta è nota sin dagli anni '80. Diversi studi hanno evidenziato una rilevante incidenza degli stati di trombofilia genetica o acquisita nelle donne affette da abortività ripetuta. Le differenti percentuali di incidenza della trombofilia nei vari studi potrebbero derivare dalla diversa etnia delle pazienti selezionate, in particolare per le varianti geniche di trombofilia ereditaria, e per i diversi criteri di inclusione e esclusione. Sono tuttavia relativamente pochi gli studi della Letteratura che hanno sottolineato una linea di condotta terapeutica per le donne trombofiliche affette da abortività ripetuta e in particolare sul potenziale ruolo terapeutico dei farmaci antitrombotici in tali pazienti. Il ruolo terapeutico delle eparine nella patologia trombotica è infatti ben affermato da numerose pubblicazioni ed è consolidato nella pratica clinica sia per la patologie trombotiche del versante arterioso sia per le patologie trombotiche del versante venoso. Un ruolo di trombo-profilassi attiva della tromboembolia venosa è inoltre sottolineato da numerose pubblicazioni disponibili in Letteratura nelle donne in gravidanza con o senza trombofilia. Soltanto negli ultimi anni poche pubblicazioni hanno sottolineato l'importanza terapeutica delle eparine nella prevenzione della abortività in donne trombofiliche poliabortive. Tali studi hanno permesso di individuare un sensibile miglioramento dell'outcome delle gravidanze di tali pazienti ad elevato rischio di abortività e patologia tromboembolica venosa.

Editorial Focus

The role of low molecular weight heparin to prevent miscarriage in thrombophilic women

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Thrombotic mechanisms underlie many pregnancy complications, in particular recurrent fetal loss (RFL). RFL has frequently been associated with thrombophilia and a hypercoagulable state (1–5). In 1999, studies by Brenner et al. identified thrombophilia as a principal cause of RFL in more than 40% of affected women (2, 3). Further studies underlined a pathogenetic role of inherited thrombophilia in women affected by RFL. Sanson et al., in fact, reported an increased frequency of antithrombin III, protein C and protein S deficiency in women with RFL (4), while several studies underlined the role of inherited thrombophilia (in particular related to factor V Leiden gene polymorphism and prothrombin A20210G gene polymorphism) in the pathophysiology of recurrent fetal loss (5, 6).

Yet, acquired thrombophilia has also been associated with RFL. A study by Dossenbach et al., in fact, revealed that elevated maternal plasma levels of clotting factor VIII tend to be associated with an increased risk of RFL (7). Moreover, several studies are available that address the association of RFL with primary or secondary antiphospholipid syndrome (8). However, increasing evidence is emerging for the role of inherited and/or acquired thrombophilia in women with *in vitro* fertilisation failure (9–10).

For this reason women with inherited and/or acquired thrombophilia besides pregnancy complications, such as RFL, may suffer from thrombosis, in particular deep venous thrombosis (DVT), and pregnancy is actually one of the well-recognised thrombotic risk factors (11). In several reports on thromboprophylaxis in asymptomatic pregnant women with or without thrombophilia (12), recommendations were made for unfractionated heparin twice daily or low molecular weight heparin once daily, in particular for high risk women [i.e. antithrombin deficiency, more than one thrombophilic abnormality, homozigosity for one thrombophilic gene polymorphism, or first-degree relative who experienced juvenile severe venous thromboembolism (VTE)] (13). Yet, thromboprophylaxis has been suggested with several regimens also for pregnant women who experienced VTE even if thrombophilia has not been already detected (13).

However, all data available in the literature seem to focus on the role of heparins to avoid VTE or late pregnancy complications such as intrauterine growth restriction, and seem not to understand the possible role of antithrombotic treatment to avoid miscarriage, although some reports also demonstrated an improvement in pregnancy outcome in thrombophilic women undergoing antithrombotic treatment with low molecular weight heparin (14). In the current issue of *Thrombosis and Haemostasis*, Sarig et al. (15) report in their study, which is a part of the LIVE-ENOX study (16), the effectiveness of low molecular weight heparin administered twice daily to thrombophilic women affected by RFL. Furthermore, the authors point out a line to modulate and to monitor the effect of low molecular weight heparin administration by several haemostatic tests, such as plasmatic total and free tissue factor pathway inhibitor (TFPI) as well as anti-Xa activity. Further detailed studies are needed to develop these topics and could emphasise avoiding thrombotic and/or haemorrhagic disorders during treatment with low molecular weight heparin twice daily in pregnant thrombophilic women.

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La sindrome da anticorpi antifosfolipidi (APS) è una delle principali forme di trombofilia acquisita ed è frequentemente associata a patologia della gravidanza, in particolare alla poliabortività del I° trimestre. Tale associazione è ancor più rafforzata dal fatto che i criteri diagnostici della sindrome stessa, secondo le linee guida della Società Americana di Reumatologia e della Società Internazionale di Trombosi ed Emostasi, includono nei criteri clinici per la diagnosi della APS la patologia della gravidanza.

La APS inoltre è frequentemente associata ad alterazione di parametri di screening dell'emostasi come l'aPTT che si può presentare prolungato in presenza di anticorpi antifosfolipidi. Gli anticorpi antifosfolipidi più frequenti nelle APS e quindi più comunemente associati alla poliabortività sono gli anticorpi anticardiolipina (classi IgG e IgM) e il lupus anticoagulant; negli ultimi anni inoltre un ruolo predominante è stato riconosciuto anche agli anticorpi anti-□2 glicoproteina I (anti□□2 GP I). Tuttavia, in alcune rare forme di APS possono essere presenti anche altri tipi di anticorpi antifosfolipidi e\o anticorpi diretti contro fattori coagulativi. Alcune pubblicazioni disponibili in Letteratura hanno infatti identificato anticorpi diretti contro il fattore XII in donne con APS. Nella nostra esperienza clinica abbiamo descritto il caso di una donna poliabortiva che presentava costantemente e contemporaneamente alterati alcuni parametri coagulativi come prolungamento dell'aPTT, bassi livelli di fattori XII, presenza di anticorpi diretti contro il fattore XII associati alla presenza sporadica e\o transitoria del LAC.

Tale associazione è stata descritta per la prima volta da un punto di vista clinico e pone l'accento sulla rilevanza clinica della gestione delle pazienti

poliabortive presentanti alterazioni dell'aPTT in quanto tale parametro può risultare allungato anche in altre condizioni quali le carenze di alcuni fattori coagulativi o in corso di trattamenti farmacologici quali quelli eparinici. La gestione clinica di tali pazienti risulta quindi particolarmente impegnativa in quanto deve presupporre un'attenta diagnosi differenziale tra le condizioni patologiche che determinano allungamento dell'aPTT legate alla trombofilia acquisita (ad esempio APS), le condizioni patologiche che determinano allungamento dell'aPTT con trend emorragico (ad esempio le carenze dei fattori coagulativi) e le condizioni iatrogene determinanti l'allungamento dell'aPTT (ad esempio i trattamenti antitrombotici con eparina non frazionata).

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Methodology

Acquired factor XII deficiency in a woman with recurrent pregnancy loss: working on a differential diagnosis in a single case

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Abstract

Background: Antiphospholipid syndrome (APS) has been often associated to RPL since 1980 and some reports in the Literature rarely described antibodies to factor XII in patients with APS.

Case history: We report the case history of 34-year-old caucasian women with recurrent fetal loss and persistent prolonged activated partial thromboplastin time. Haemostatic tests revealed persistent light decrease of clotting factor XII with normal values of IgG and IgM anticardiolipin antibodies and transient positivity for lupus anticoagulant (LA). Few reports in the Literature described antibodies to factor XII in patient with antiphospholipid syndrome (APS) and transient LA. So, once other causes of RPL were excluded, the patient was diagnosed an unusual form of APS associated to antibodies to factor XII, reduced factor XII plasma levels, transient LA and prolonged activated partial thromboplastin time.

Discussion: We suggest to consider also antibodies directed to clotting factors (e.g. factor XII in our case) as second step of thrombophilia screening in RPL, in particular if a persistent prolonged aPTT is present without an apparent cause.

Background

Recurrent pregnancy loss (RPL) is one of the most common cause of sterility. An our recent study underlined the relevant role of d-dimer, as marker of hypercoagulable state, to identify thrombophilia in women affected by primary or secondary sterility [1]

In 1999 a study written by Brenner et al. identified thrombophilia as principal cause of more than 40% of women affected by RPL [2]. Further studies underlined a pathogenetic role of inherited thrombophilia in women affected by RPL. Sanson et al., in fact, reported an increased frequency of antithrombin III, protein C and protein S deficiency in women with RPL [3], while Grandone et al. found an increased incidence of factor V Leiden in women with unexplained RPL [4]. Also prothrombin A20210G gene polymorphism has been reported as possible cause of RPL in several studies [2,5,6].

Yet, also acquired thrombophilia has been associated to RPL. A study by Dossenbach et al., in fact, revealed that elevated maternal plasma levels of clotting factor VIII tend

Table 1: Screening for disorder of haemostasis in the patient with RPL.

Parameters (unit of measurement)	Results	Normal range
Prothrombin time (INR)	1.15	0.8–1.2
Activated partial thromboplastin time (ratio)	1.45	0.8–1.2
Fibrinogen (mg/dL)	275	220–420
Protein C (%)	93	60–125
Protein S (%)	92	60–125
Antithrombin III (%)	104	80–120
Anticardiolipin Ab IgM (U/GPL)	1.7	< 2.0
Anticardiolipin Ab IgG (U/MPL)	3.9	< 7.0
β-2-GP I Ab	Absent	Absent
Lupus anticoagulant	Absent	Absent
Factor XII (%)	65	80–120
Factor XI (%)	113	80–120
Factor X (%)	112	80–120
Factor IX (%)	99	80–120
Factor VIII (%)	88	65–155
Factor V (%)	110	80–120
MTHFRC677T gene polymorphism	Wild type	Wild type
PTHRA20210 gene polymorphism	Wild Type	Wild type
FVL gene polymorphism	Wild Type	Wild type

INR: international normalised ratio
MTHFRC677T: methylene-tetra-hydrp-folate reductase C677T gene polymorphism
PTHRA20210G: prothrombin A20210G gene polymorphism
FVL: factor V Leiden gene polymorphism
β-2-GP I Ab: Antibodies to β-2-glycoprotein I

to be associated to an increased risk of RPL [7]. Moreover, several studies in the Literature are available for the association of RPL and primary or secondary antiphospholipid syndrome [8,9]. On this topic also a rare condition as acquired deficiency of clotting factor XII has been described. Braulke et al. identified for the first time a factor XII deficiency in RPL [10], but subsequently Jones et al. reported acquired factor XII deficiency in a subpopulation of women with antiphospholipid antibodies and RPL [11-13]. We here report a really interesting case report of woman affected by unexplained RPL, prolonged activated partial thrombplastin time and mild/moderate reduction of clotting factor XII.

Case presentation

A 34-year-old Caucasian non smoking woman was referred to our Sterility Center. Her personal anamnesis revealed three early pregnancy loss within 8 and 12 week of gestation and one extrauterine pregnancy. The patient did not revealed previous thromboembolic disease (arterial or venous) nor haemorragic disorders; moreover patient was not ongoing any type of pharmacological treatment. A thorough familial anamnesis did not show a trend toward thromboembolic and/or haemorragic disease.

To understand pathophysiology of her RPL the patient performed several laboratory and instrumental tests.

A normal ovarian function and ovulation were detected by normal values of Follicle-stimulating Hormone, Luteinising Hormone, oestradiol and progesterone and by ovarian ultrasound scan (data not shown). Uterine and salpinxes malformation was excluded by hysterosalpingography and hysteroscopy (data not shown). Endocrinological diseases such as diabetes and dysthyroidism were evaluated and excluded by normal values of glycaemia, thriiodothyronine (i.e. FT3), thyroxine (i.e. FT4) and Thyroid-stimulating Hormone (data not shown). Inflammatory chronic diseases were excluded by normal values of erythro-sedimentation rate and acute phase C reactive protein and immunopathological chronic disease, such as erytematosus systemic lupus, by normal level of antinuclear antibodies (ANA), anitmithocondrial antibodies (AMA) and smooth muscle antibodies (SMA) too (data not shown); moreover patient did not suffer of chronic joint paint or fever or other related symptoms.

Yet, routine haemostatic tests showed normal value of prothrombin time, measured as International Normalised Ratio (PT INR, 1.15) and a prolonged activated thromboplastin time, measured as ratio (aPTT, 1.45) (table 1). To confirm this laboratory alteration, after 15 days a second step of haemostatic parameters were tested and confirmed normal value of PT INR and prolonged aPTT (1.46, table 1 and 2), associated to normal levels of anticardiolipin antibodies (tested by an ELISA method; IgM 1.7 U/MPL

Table 2: Monitor of the alteration of haemostasis in the patient with RPL.

Parameters (Unit of measurement)	First screening	Second screening	Third screening	Fourth screening	Normal value
aPTT (ratio)	1.45	1.36	1.28	1.44	0.8–1.2
Factor XII (%)	65	50	55	43	80–120
Anticardiolipin Ab IgM (U/MPL)	1.7	1.3	1.5	1.5	< 2.0
Anticardiolipin Ab IgG (U/GPL)	3.9	5.4	3.8	2.5	< 7.0
β-2-GP I Ab	Absent	Absent	Absent	Absent	Absent
Lupus anticoagulant	Absent	Absent	Present	Absent	Absent
Anti-factor XII Ab	Not tested	Not tested	Not tested	Present	Absent
Antinuclear antibodies (ANA)	**Absent**	**Not tested**	**Not tested**	**Absent**	**Not tested**

aPTT: activated partial thromboplastin time
ab: antibodies
β-2-GP I Ab: Antibodies to β-2-glycoprotein I

and IgG 3.9 U/GPL) (table 1 and 2), fibrinogen and clotting factors V, VIII, IX, X, XI (table1), while a reduced plasmatic level of clotting factor XII was detected (65%, table 1 and 2). So, we tested for aPTT and factor XII first degree relatives (i.e. one sister and parents) but did not find alterations (data not shown). Yet, lupus anticoagulant and antibodies to β-2-glycoprotein I were absent (table 1 and 2). Furthermore, because RPL was associated to reduced clotting inhibitors also protein C, protein S and antithrombin III were analysed and resulted in normal range (table 1) such as inherited thrombophilia associated to Factor V Leiden and prothrombin A20210G gene polymorphisms (table 1). Moreover, to confirm factor XII deficiency, after one month the patient was evaluated again and results showed again prolonged aPTT (1.48), reduced factor XII (55%). So, we tested aPTT adding to the plasma patient's a normal plasma sample with a ratio of plasma patient's to pool plasma 1/1 v/v; results revealed a partial correction of aPTT (1.30) suggesting a possible presence of an acquired clotting inhibitor and/or antiphospholipid antibodies. A new evaluation of common antiphospholipid antibodies began with normal levels of anticardiolipin antibodies (tested again with an ELISA method; IgM 1.5 and IgG 3.8) and absence of antibodies to β-2-Glycoprotein I, but a positivity for lupus anticoagulant was detected (table 2). Lupus anticoagulant was assayed according to recommendations of the International Society of Thrombosis and Haemostasis. Furthermore, at this time we tested again ANA that resulted again negative. Finally, because a transient positivity of lupus anticoagulant has been associated with factor XII deficiency in patients with antiphospholipid syndrome in rare cases [14,15], after one month the patient was evaluated one more time and in this time also antibodies to clotting factor XII were evaluated by an ELISA method. Results showed prolonged aPTT (1.44), reduced factor XII plasma levels (43%), normal range of anticardiolipin antibodies (IgM and IgG), absence of antibodies to β-2-Glycoprotein I, negativity for lupus anticoagulant, while antibodies to factor XII have been detected (table 2). So, according to our data and data available in the Literature the patient was diagnosed an unusual form of primary antiphospholipid syndrome and an antithrombotic treatment was suggested.

Discussion

Inherited factor XII deficiency seems to be not associated to bleeding tendency if referred to major surgery [16]. However, some individual with reduction of factor XII seems to have a trend toward thrombosis [16], but pathophysiological mechanisms underlying should be better understood. Therefore, deficiency of clotting factor XII has been reported as also thrombotic risk factor and Halbmayer et al. suggested to consider factor XII deficiency in patients with recurrent thromboembolism [17]. One of the possible mechanisms could be associated to the presence of acquired clotting inhibitors eventually associated to the presence of an antiphospholipid syndrome [18]. Acquired clotting inhibitors, in fact, have been identified in several disease and frequently are associated to a thrombophilic trend [19].

Furthermore, from a clinical point of view, alteration of haemostasis with a trend toward thrombophilia has been frequently associated to RPL [2,8]. According to a multivariate analysis on the etiology of thrombophilia by Yamada et al., clotting factor XII deficiency has been found in 4.2% of women affected by RPL [20]. Another interesting study by Iinuma et al. underlined clotting factor XII activity and not its 46C/T gene polymorphism as cause of RPL in a selected population [21]. However, factor XII deficiency may be due to inherited deficiency or acquired deficiency during an acquired disease such as antiphospholipid syndrome. In this last condition, in fact, we may find an acquired factor XII deficiency because the presence of antibodies to factor XII; moreover antibodies to factor XII may be present during antiphospholipid syndrome alone or together to lupus anticoagulant [11]: antibodies to factor XII have been described, in fact, also in patients with antiphospholipid syndrome and transient

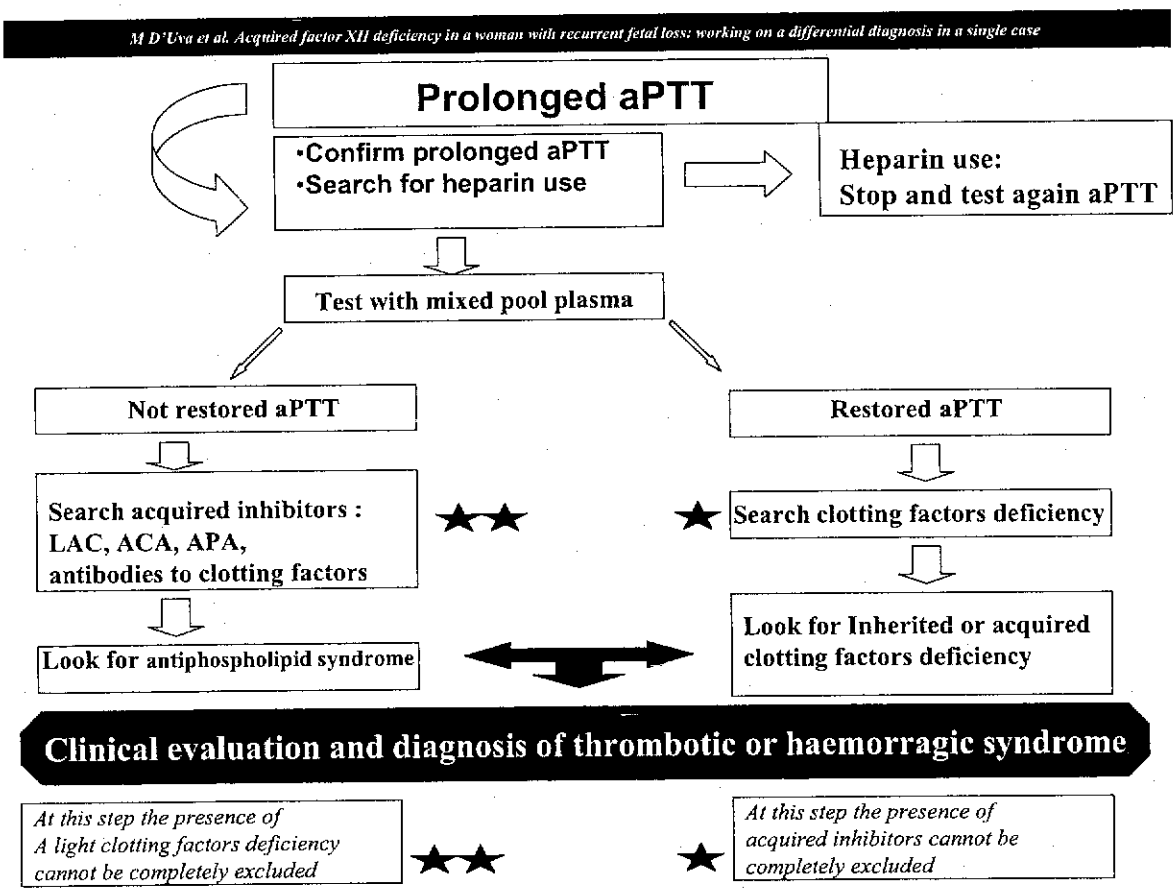

Figure 1
text

lupus anticoagulant [14,15]. Moreover, Jones et al. described that antibodies to factor XII can be present in women with RPL and antiphospholipid syndrome more than anticardiolipin antibodies and antibodies to β-2-glycoprotein I [11]. So, according to these data, antibodies to factor XII may be implicated in the pathophysiology of the hypercoagulable state in women with antiphospholipid syndrome showing RPL and their incidence in this clinical setting could be underestimated according to the data available from the Literature [18]. This hypothesis is really intriguing also in the case we described.

The reported patient affected by RPL, in fact, did not show an apparent cause of RPL other than reduction of clotting factor XII. Also laboratory tests, concerning thrombophilia, did not show common alteration of haemostasis associated to RPL such as factor V Leiden gene polymorphism or reduction of clotting inhibitors (i.e. protein C, protein S, antithrombin III). Yet, the clinical presentation and the presence of persistent prolonged aPTT suggested periodical tests concerning possible clotting factors deficiency and/or the presence of antiphospholipid syndrome. Results of this screening confirmed us the partial and acquired factor XII deficiency, due to the presence of antibodies to factor XII, in presence of transient lupus anticoagulant.

So, in conclusion we suggest to search in such cases also antibodies directed to clotting factors (e.g. factor XII in our case) as second step of thrombophilia screening in RPL, in particular if a persistent prolonged aPTT is present (figure 1) without an apparent cause.

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5. P Di Micco, **M D'Uva.** Ovarian vein thrombosis and its relevance in the daily clinical management for hospitalised patients. Thromb Haemost 2006; **96**: 109-110. [Editorial Comment]

La patologia trombotica venosa è una patologia multifattoriale che riconosce fattori predisponenti congeniti e acquisiti. I fattori predisponenti congeniti sono ben noti e legati a varianti geniche di alcuni fattori della coagulazione come il fattore V Leiden e la protrombina A20210G o la variante genica C677T della MTHFR condizionante iperomocisteinemia e ipercoagulabilità; altre condizioni congenite di trombofilia sono le carenze di anticoagulanti fisiologici quali proteina C, proteina S e antitrombina III. Le condizioni di rischio acquisite per la trombosi venosa si dividono a loro volta in condizioni molecolari, quali la sindrome da anticorpi antifosfolipidi o gli aumenti del fattore VIII o la resistenza alla proteina C attivata per cause non genetiche, e condizioni di rischio per trombosi venosa non molecolari, rappresentate da patologie maligne, dall'uso di estroprogestinici, dalla chirurgia e dall'ipomobilità post-operatoria, dalla gravidanza e dal puerperio. Nonostante una profonda conoscenza dei fattori di rischio per la trombosi venosa profonda, una percentuale variabile di casi risulta essere idiopatica e coinvolge inoltre anche distretti venosi atipici come quello splancnico e quello ovarico. Una reale conoscenza dell'incidenza di trombosi venose ovariche non è ben nota neanche dagli ultimi studi presenti in Letteratura. Alcuni dati rilevanti tuttavia emergono dalle ultime pubblicazioni come la frequente associazione tra trombosi ovariche e post-partum\puerperio tanto in caso di parto spontaneo che di taglio cesareo. Una minore incidenza di tali eventi trombotici è stata invece riscontrata durante la gestazione mentre sporadiche osservazioni sono state riportate in corso di sindrome da anticorpi antifosfolipidi. Il ruolo della trombofilia genetica sembra invece essere di minore rilevanza secondo i dati già presenti in Letteratura.
Il problema clinicamente rilevante in tali condizioni sembra tuttavia essere legato a un tempestivo approccio diagnostico in quanto, frequentemente, le

trombosi venose ovariche risultano difficilmente diagnosticabili tramite le normali procedure di diagnostica strumentale quale l'ecocolor-Doppler e necessitano quindi frequentemente di un approccio diagnostico per imaging di diversa natura quale angioTC e\o angioRMN. Il problema è piuttosto rilevante da un punto di vista clinico e dal punto di vista prognostico in quanto le trombosi venose ovariche si associano ad un notevole tasso di embolizzazione polmonare con i relativi rischi quoad vitam e quoad valetudinem delle pazienti affette.

Editorial Focus

Ovarian vein thrombosis and its relevance in the daily clinical management for hospitalised patients

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Ovarian vein thrombosis (OVT) is considered a rare event mainly associated with the puerperium or with gynaecological malignancy (1). Because OVT is an abdominal deep venous thrombosis (DVT), we may also consider it as an unusual site of thrombosis, and for this reason epidemiological data focusing on its real incidence are lacking in the literature. Frequently, we may find published data about OVT as clinical case reports or short series of selected patients.

From a pathological point of view, OVT is usually associated with infective complications and\or sepsis, or with the possibility of subsequent pulmonary embolism, causing life-threatening complications in both situations (2). Yet, a relevant problem in the management of OVT is related to the fact that OVT is frequently asymptomatic, in particular at the beginning of the disease. For this reason, often the pulmonary embolism event may be the first clear clinical sign of an underlying OVT.

While previous reports on patients affected by OVT failed to demonstrate an association between OVT and inherited thrombophilia (3), acquired thrombotic risk factors seem to be more commonly associated with OVT (4–6). Moreover, this relation resembles an association with other types of abdominal vein thrombosis, e.g. mesenteric venous thrombosis or portal vein thrombosis. As such, acquired thrombotic risk factors for OVT should be available and well known in order to point out a correct clinical suspect of OVT, the clear association between OVT and puerperium (7) or gynaecological malignancy may predetermine the successful management in particular if a surgical approach is needed (8). Further associations of OTV with acquired prothrombotic conditions such as nephrotic syndrome (9), antiphospholipid syndrome (10) or chronic haematological syndrome (essential thrombocythaemia) (11) have been reported as well. In the daily clinical management adequate knowledge of OVT risk factors is relevant because the right ovarian vein appears to be more commonly involved, and the right lower quadrant abdominal pain associated with fever may be present during OVT. Thus, a correct differential diagnosis with appendicitis, pyelonephritis or endometritis should be made. Ultrasound scan, also associated with colour-Doppler examination, does not represent the golden standard for OVT and an additional imaging approach is needed to confirm OVT diagnosis with magnetic resonance or mainly with CT scan (12).

In this issue of *Thrombosis and Haemostasis*, Wysokinska et al. **(see pages 126-31)** report their experience with OVT through a retrospective analysis of all recorded OVT at the Mayo Clinic from 1990 to 2006 (13). Their data not only confirm a more common association between OVT and acquired thrombotic risk factors if compared with inherited thrombophilia, but also give valuable information concerning the follow-up of their OVT patients. A poor prognosis of patients with OVT is linked to the presence of an underlying malignancy. Yet, data concerning the similar involvement of left and right ovarian veins in OVT imply that this kind of thrombosis is underestimated and underdiagnosed. Although their data are derived from a retrospective analysis, the present article can improve our knowledge on the natural history of OVT particularly considering OVT as possible cause of pulmonary embolism.

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6. B Oliviero, P Di Micco, G Guarino, I de Sio, **M D'Uva**, S Gentile. A case of thrombosis of the superior mesenteric vein occurring in a young woman taking oral contraceptives: full and fast resolution with low molecular weight heparin. Clin Lab 2007; 53: 167-171 [Case report]

La patologia trombotica venosa è una patologia multifattoriale che riconosce fattori predisponenti congeniti e acquisiti. Nonostante una buona conoscenza dei fattori di rischio per la trombosi venosa profonda una percentuale coinvolge distretti venosi atipici come quello splancnico. Tra i fattori di rischio acquisiti per la patologia trombotica venosa un ruolo ben noto è esercitato dalle terapie estroprogestiniche a scopo anticoncezionale. I contraccettivi di terza generazione sembrano essere maggiormente a rischio per tali patologie rispetto a quelli di seconda generazione. Dal punto di vista clinico nella maggioranza dei casi le manifestazioni trombotiche sono a carico del distretto venoso profondo degli arti inferiori, tuttavia un discreto numero di eventi trombotici venosi si manifesta a carico di distretti venosi atipici quali quello retinico, quello cerebrale e quello splancnico. Riportiamo la nostra esperienza clinica nella diagnosi precoce di trombosi venosa profonda della vena mesenterica superiore nel suo tratto prossimale in una giovane donna in trattamento contraccettivo venuta all'osservazione per un dolore addominale atipico che non regrediva con la somministrazione di antispastici e antiacidi. Un progressivo peggioramento della sintomatologia, la mancata risposta alla terapia farmacologica associata alla comparsa di diarrea e alla normalità dei comuni test di laboratorio suggerì l'esecuzione di un ecografia addominale che evidenziò la presenza di una trombosi della vena mesenterica superiore. L'assenza di segni clinici di addome acuto, la normalità dei test di laboratorio in particolare l'assenza di indici di citolisi e l'assenza di leucocitosi ci hanno condotto a un tentativo di trattamento conservativo farmacologico basato sul monitoraggio clinico-laboratoristico e sulla somministrazione di eparina a basso peso molecolare a dosi terapeutiche (i.e. 100 U\Kg bid). Un rapido e progressivo miglioramento del

quadro clinico e laboratoristico veniva confermato a pochi giorni di distanza dall'esecuzione di una nuova ecografia addominale che confermava la risoluzione della trombosi descritta. Un approfondimento diagnostico molecolare non ha evidenziato alcun tipo di predisposizione molecolare di tipo genetico o acquisito nella paziente descritta ponendo quindi ancora una volta l'attenzione sul ruolo protrombotico dei contraccettivi orali. Nel nostro caso l'esecuzione precoce di un'ecografia addominale ha permesso una diagnosi precoce e un trattamento conservativo di tipo farmacologico che ha evitato un approccio chirurgico.

Clin. Lab. 2007;53:167-171

CASE REPORT

A Case of Thrombosis of the Superior Mesenteric Vein Occurring in a Young Woman Taking Oral Contraceptives: Full and Fast Resolution with Low Molecular Weight Heparin

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SUMMARY

Mesenteric venous thrombosis (MVT) is an unusual site of deep venous thrombosis. Little is known about risk factors of MVT, but available data seem to confirm a pathogenetic role of acquired thrombotic risk factors as well as inherited thrombotic risk factors. However, few cases on the association of MVT with oral contraceptive use have been described. We here report a case of MVT in a woman on oral contraception with fine and complete resolution after a fast diagnosis with abdominal ultrasound imaging and prompt therapy based on low molecular weight heparin. (Clin. Lab. 2007;53:167-171)

KEY WORDS

oral contraceptives, DIANE©, mesenteric venous thrombosis, deep venous thrombosis, hypercoagulable state, thrombophilia, women's health, abdominal thrombosis

Background

Thrombosis of the mesenteric vein (MVT) is a clinical picture quite different from mesenteric arterial thrombosis and responsible for frequent cases of bowel infarction (1-2). The known frequency of MVT is about 5-10% out of all acute mesenteric ischemias according to data reported by Brandt and Boley (3). Usually, a large collateral venous circulation arises during MVT occurrence, and it prevents bowel infarction. In fact, this late clinical setting represents nearly 1 out of 1000 surgical abdominal emergencies (4). Several conditions may be associated with clinical evidence of venous thrombosis such as deficiency of protein C, protein S, antithrombin as well as activated protein C resistance, primary or secondary antiphospholipid syndrome, hyperhomocysteinemia, inherited thrombophilia for the presence of factor V Leiden, prothombin G20210A gene polymorphism, and methlyene-tetrahydrofolate-reductase C677T gene polymorphism (1,3,5-6). In addition, several of the above mentioned conditions are thrombotic risk factors and markers of a hypercoagulable state. Furthermore, other clinical pictures may trigger a hypercoagulable state and may induce MVT, such as malignancy (7-8), liver cirrhosis and/or portal hypertension, thrombocytosis (in particular essential thrombocythemia), polycythemia vera (3), oral contraceptive use (9-13), pregnancy, post-surgical state, trauma, inflammatory diseases (i.e. pancreatitis, peritonitis, abdominal abscesses, inflammatory chronic bowel diseases) (3). Conversely, venous thrombosis after suspension of an antithrombotic treatment is infrequently observed (15-16). Finally, common thrombotic risk factors for deep venous thrombosis must be also regarded as risk factors for MVT (3).

From a clinical point of view we may distinguish two clinical pictures: subacute and acute MVT. Acute MVT

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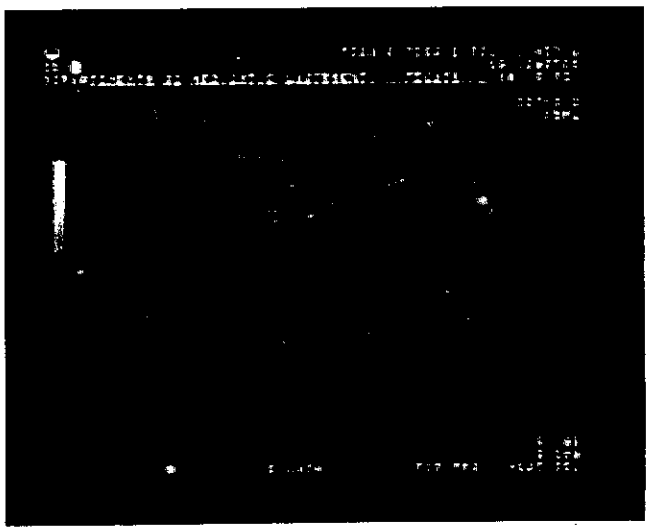

Figure 1: Ultrasound imaging of a floating hypoechoic thrombus localized in the superior mesenteric vein.

Table 1: Laboratory findings of the woman affected by MVT

Test (unit of measurement)	Value	Normal range
β-HCG (mU/L)	0	<10
CRP (ng/dL)	< 0.5	0.0-0.5
ESR at first hour (mm)	15	< 20
ALT aminotransferarse (U/L)	16	11-40
AST aminotransferase (U/L)	11	11-40
Amylase (U/L)	38	30-110
Creatinine (mg/dL)	0.8	0.7-1.1
LDH (U/L)	380	290-615
BUN (mg/dL)	14	8-50
Total bilirubin (mg/dL)	0.48	0.20-1-10
Alkaline phosphatase (U/L)	54	38-120
Prothrombin time (INR)	1.01	0.8-1.2
Activated partial thromboplastin time (ratio)	0.85	0.8-1.2
Fibrinogen (mg/dL)	334	220-420
D-dimer test	Positive	Negative
Hemocrome /blood count		
RBC (cell/mm^3)	4.1 x 10^6	4.0-5.5 x 10^6
WBC (cell/mm^3)	6.5 x 10^3	4.5-10.0 x 10^3
Hb (g/dL)	13	12-16
Hct (%)	39.1	37-45
MCV (fL)	94.2	80-96
MCH (pg)	31.0	27-31
MCHC (g/dL)	32.5	32-36
PLT (cell/mm^3)	278 x 10^3	130-400 x 10^3

β-HCG: beta human chorionic gonadotropin
CRP: acute phase C-reactive protein
ESR: erythrocyte sedimentation rate
ALT: alanine aminotransferase
AST: aspartate aminotransferase
BUN: blood urea nitrogen
INR: international normalized ratio
RBC: red blood cells
WBC: white blood cells
Hb: hemoglobin
Hct: hematocrit
MCV: mean corpuscular volume
MCH: mean corpuscular hemoglobin
MCHC: mean corpuscular hemoglobin concentration
PLT: platelets

is frequently associated with severe abdominal pain, nausea, vomiting, upper and/or lower gastrointestinal bleeding, fever and, in a few cases, septic shock. Because of an efficacious collateral circulation, symptoms are mild in subacute MTV (abdominal pain, nausea, vomiting and presence of red blood cells in the stool) and the differential diagnosis of irritable bowel disease is difficult to be achieved. Nevertheless, the pathophysiology of MVT is still unclear, the clinical presentation is frequently shifty, and the diagnosis is based on imaging techniques such as ultrasound scan (US), computed tomography (CT) and magnetic resonance imaging (MRI) (3, 17). Frequently, laparoscopy and/or laparotomy are needed to achieve a firm diagnosis of MVT (3).
Here we report an interesting case of thrombosis of the superior mesenteric vein occurring in a young woman who had taken oral contraceptives and in whom diagnosis of MVT was achieved by US technique.

Case report

A 31-year-old, non-smoking Caucasian woman (h: 155 cm, w: 52 kg, BMI: 20 kg/m^2) was referred to our division of Internal Medicine for a recent episode of abdominal pain. After lunch, the patient felt a severe and sudden upper abdominal pain, which lasted continuously for nearly two hours and then spontaneously ceased. Nausea, vomiting, and fever were absent. Pain did not stop with auto-medication based on antacids. After three hours a mild diarrhea appeared, associated with a new occurrence of sudden and severe abdominal pain localized to the right hypochondrium.
The patient's history revealed a previous irritable bowel syndrome. Previous arterial and/or venous thrombotic disorders or pregnancy loss were not recorded. Two live birth babies were, in fact, delivered after regular pregnancies. The patient had taken oral contraceptives (ethinyl estradiol 0.035 mg and cyproterone 2 mg for each tablet, DIANE©) for the previous six years, since she was 25 years old, and she did not report side effects. Moreover, a thorough anamnesis revealed that the patient had recently discontinued (nearly four days ago) the pill (ethinyl estradiol 0.035 mg and cyproterone 2 mg for each tablet, DIANE©).
Physical examination revealed only abdominal pain localized in the mesogastrium and right iliac fossa, without Blumberg's sign. A thorough gynecological examination excluded extrauterine pregnancy (according also to beta-HCG level). Laboratory and instrumental tests were provided and are reported below. Electrocardiogram, thoracic and abdominal X-ray examination did not reveal any alteration. Standard laboratory tests (i.e. RBC and WBC counts, hemoglobin, platelet counts, aminotransferases, amylase, alkaline phosphatase, BUN, creatinine, serum electrolytes, LDH, total bilirubin,

Table 2: Thrombophilia tests used to screen the patient with MVT

Thrombophilic test (unit of measurement)	Results	Normal range
Prothrombin time (INR)	1.01	0.8-1.2
Activated partial thromboplastin time (ratio)	0.85	0.8-1.2
Fibrinogen (mg/dL)	334	220-420
Protein C (%)	94	60-125
Protein S (%)	88	60-125
Antithrombin III (%)	107	80-120
Activated protein C resistance	0.98	>0.77
Anticardiolipin Ab IgM (U/GPL)	1.7	< 2
Anticardiolipin Ab IgG (U/MPL)	3.9	< 7
Lupus anticoagulant	Absent	Absent
Homocysteine (µmol/L)	9	5-15
MTHFRC677T gene polymorphism	Heterozygosity	wild type
PTHR G20210A gene polymorphism	wild type	wild type
FVL gene polymorphism	wild type	wild type

INR: international normalized ratio
MTHFRC677T: methylenetetrahydrofolate reductase C677T gene polymorphism
PTHR G20210A: prothrombin A20210G gene polymorphism
FVL: factor V Leiden gene polymorphism

acute phase C-reactive protein, erythrocyte sedimentation rate (ESR), prothrombin time, activated partial thromboplastin time, fibrinogen) did not show any alteration and are reported in Table 1. An abdominal ultrasound scan was performed and showed normal size and echostructure of the kidneys, liver, pancreas, and spleen; portal and splenic vein showed normal diameter and flow at color-power Doppler. Superior mesenteric vein scan revealed thrombosis with a floating thrombus (Figure 1). To have a laboratory confirmation of this diagnosis a D-dimer test was also performed and showed increased D-dimer (see Table 1).

Because physical examination, laboratory data (including LDH as specific markers of cytolysis in these clinical settings) and instrumental data did not reveal an acute surgical abdomen, a pharmacological treatment of MVT based on high doses of low molecular weight heparin (enoxaparin 6000 U, twice daily) was immediately started. Antithrombotic treatment with LMWH went on for two weeks before overlapping oral anticoagulation based on warfarin administration according to INR range 2.0-3.0. A new abdominal ultrasound scan was then performed and showed the disappearance of MVT. Before oral anticoagulation was started a laboratory study to detect possible inherited and/or acquired thrombophilia was performed (i.e. deficiency of protein C, protein S, AT III, activated protein C resistance, MTHFR C677T gene polymorphism, factor V Leiden gene polymorphism, prothrombin A20210G gene polymorphism and the presence of anticardiolipin antibodies, lupus anticoagulant) and did not reveal alterations (Table 2). Thus, also thrombophilia screening did not underline further thrombotic risk factors. Therefore, the only identified thrombotic risk factor seemed to be the oral contraceptive use. After ten months, the patient is still taking oral anticoagulation and is doing well.

DISCUSSION

The association between oral contraceptives and venous thrombosis has been known since the 1960s. Even though prothrombotic action has been attributed to estrogen (18), subsequent studies underlined a relevant role also for the type of progesterone (19). In particular, recent data have shown that third generation oral contraceptives seem to carry a higher risk for DVT (13, 20).

From a molecular point of view oral contraceptive use allows a trend toward a hypercoagulable state because of an increase of clotting factor synthesis (i.e. clotting factors VII, VIII, IX, X, XI, fibrinogen and prothrombin) (21) but this increase seems to be more relevant for factor VII. Moreover, also an acquired form of activated protein C resistance has been recorded during oral contraceptive use (22). Furthermore, the association of oral contraceptive use and inherited and/or acquired thrombophilia strongly increases the risk of DVT (23-24). However, although the risk of DVT in oral contraceptive users has been well established for deep venous thrombosis, alone or associated with pulmonary embolism (11-14, 18-20), and for cerebral venous thrombosis (25), little is known about the risk of MVT and oral contraceptive use. Only 26 cases of MVT in oral contraceptive users have been described in the literature according to the data reported by Hassan (26).

The case we report shows an early diagnosis of MVT in a young woman who used an oral contraceptive and reported a sub-continuous abdominal pain. After exclusion of a surgical abdomen and the more common causes of abdominal pain, an early approach by ultrasound was performed also to screen abdominal vessels in order to evaluate a possible MVT. But this kind of clinical approach may be difficult in the daily clinical management because of bowel gas, timing of the ultrasound scan, and possible distal localization of thrombosis along the entire superior mesenteric vein. However, when a MVT diagnosis has been reached by ultrasound, other tests may also be avoided (i.e. computed tomography, magnetic resonance imaging) (17), in particular in patients who do not show clinical signs of surgical abdomen or bowel infarction and who do not require surgery.

In our case, in fact, the early diagnosis of MVT by ultrasound allowed an early antithrombotic treatment based on LMWH. Other reported cases of MVT described also other pharmacological therapeutic approaches (i.e. thrombolysis, i.v. heparin followed by

Table 3: Summary of reported cases of MVT and their pharmacological management with antithrombotic drugs.

Author	Antithrombotic drugs	Outcome
Reed et al. (1963)	None	Death
Lowry et al. (1969)	Dextran and UFH	Survived
Miller (1970)	None	Survived
Civetta et al. (1970)	None	Death-PE
Hurwitz et al. (1970)	None	Survived
Hurwitz et al. (1970)	None	Survived
Hurwitz et al. (1970)	None	Survived
Rose (1972)	UFH and OAT	Death
Rose (1972)	Unknown	Death
Rose (1972)	None	Death
Rose (1972)	OAT	Survived
Rose (1972)	Dextran and UFH	Survived
Ruoff et al. (1973)	UFH and OAT	Survived
Ellis et al. (1973)	None	Survived
Ellis et al. (1973)	None	Survived
Collier et al. (1975)	UFH and OAT	Survived
Milne et al. (1976)	UFH, OAT and dipyridamole	Survived
Nesbit et al. (1977)	UFH and OAT	Survived
Hoyle et al. (1977)	None	Survived
Lescher et al. (1977)	Dextran	Survived
Khodadadi et al.(1980)	UFH	Survived
Capron et al. (1981)	UFH	Death
De Stefano et al. (1982)	None	Death
Naraynsignh et al. (1984)	UFH	Death
Grubard et al. (1987)	None	Survived
Rahmouni et al. (1992)	UFH and OAT	Survived
Foo et al. (1996)	UFH	Survived
Hassan (1997)	UFH and OAT	Survived
Mitani et al. (1999)	UFH and ASA	Survived
Bonariol et al. (2000)	UFH	Survived
Tateishi et al . (2001)	UFH and thrombolysis	Survived
Kato et al. (2002)	UFH and UK	Survived
Sturm et al. (2003)	UFH	Survived
Echtibi et al. (2003)	UFH and OAT	Survived
Dager et al. (2004)	UFH, argatroban and OAT	Survived
Oliviero et al. (2006 (current report)	LMWH and OAT	Survived

UFH: unfractionated heparin
OAT: oral anticoagulation
PE: pulmonary embolism
UK: urokinase
ASA: acetylsalicylic acid
LMWH: low molecular weight heparin

oral anticoagulation) but in the last years we have observed an increase of LMWH administration in thrombotic disorders because LMWH showed safety also in cases of pulmonary embolism (27-28). However, we report in Table 3 a summary of other reported cases of MVT in which also further thrombotic risk factors other than the pill were present, and indicated the pharmacological management with antithrombotic drugs. As shown in Table 3 and in a series of abdominal vein thrombosis, we found several differences in the literature for the pharmacological antithrombotic approach, and LMWHs are less used than other drugs; this variety of possibilities is due to difficulties in the clinical approach and differential diagnosis, depends on a variety of molecular and clinical conditions associated with the diagnosis of MVT, depends on simultaneous involvement of further abdominal veins (e.g. portal vein and/or splenic vein), depends on the different timing of the diagnosis of MVT, and depends on the decision for antithrombotic pharmacological treatment or surgical treatment. Yet, the favorable outcome of our case seems to confirm the valuable therapeutic role of LMWH also in the early treatment of MVT.

In conclusion, based on this case, we noted several interesting and educational issues such as

1. *confirmation of the strong thrombophilic action of the contraceptive pill,* also without known other forms of thrombophilia, that may induce also severe and rare deep venous thrombosis in an unusual site (e.g. abdominal veins)
2. *the strong suggestion to perform a fast abdominal ultrasound scan in subjects with severe abdominal pain of unknown origin* without response to common drugs (e.g. antiacids, NSAIDs, spasmolytic drugs) who are taking a contraceptive pill in order to perform a differential diagnosis of several diseases
3. *the early identification and pharmacological treatment of MVT is necessary* in order to avoid surgical complications (e.g. bowel infarction and/or acute abdomen).

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7. P Di Micco, **M D'Uva**, I Strina, G De Placido, R Di Fiore, S Quaranta, G Castaldo. Recurrent pregnancy loss and thrombophilia. Clin Lab 2007; 53: 309-314 [Review]

Gli stati trombofilici, sia di origine congenita, sia di origine acquisita, sia di origine combinata, sono frequentemente stati associati alla abortività ricorrente e alla patologia trombotica venosa in gravidanza. Nella review vengono esaminati diversi aspetti clinici e diagnostici di tale associazione. In una prima sezione vengono riportati i dati relativi all'associazione tra trombofilia genetica e poliabortività e in particolare i diversi reports presenti in Letteratura che descrivono le condizioni di trombofilia genetica più frequentemente associate all'abortività ripetuta quali il polimorfismo del fattore V Leiden, della protrombina A 20210 G, e i deficit degli anticoagulanti naturali quali proteina C, proteina S e antitromboina III. In una diversa sezione l'iperomocisteinemia, associata o meno alle varianti geniche C677T della MTHFR, e il suo ruolo nell'indurre iperocagulabilità e abortività ripetuta vengono esplorate in accordo con i dati presenti in Letteratura. In un'ulteriore sezione vengono esaminati i dati relativi all'associazione tra abortività ripetuta e trombofilia acquisita per la presenza di sindrome da anticorpi antifosfoilipidi, incrementi di fattore coagulativo VIII e resistenza alla proteina C attivata per cause differenti dalla presenza del fattore V Leiden. Nelle ultime sezioni la review considera il ruolo dei farmaci antitrombotici, in particolare le eparine, tanto l'eparina non frazionata quanto le eparine a basso peso molecolare nella sorveglianza e nella prevenzione della trombo-embolia venosa in gravidanza e nella prevenzione dell'abortività ripetuta in donne trombofiliche.

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REVIEW

Recurrent Pregnancy Loss and Thrombophilia

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SUMMARY

In the last decades we found many data concerning the association between a hypercoagulable state and its causes and adverse pregnancy outcome, in particular recurrent pregnancy loss (RPL). Although first studies were focused only on the association between thrombophilia and RPL, subsequent studies underlined also a potential role of antithrombotic treatment to prevent vascular complication such as venous thromboembolism (VTE) during pregnancy. Now, emerging data seem to be available also on the role of active thromboprophylaxis with heparin and pregnancy outcome. This review will be focused on the recent knowledge between thrombophilia, hypercoagulable state, RPL, VTE and future perspectives. (Clin. Lab. 2007;53:309-314)

INTRODUCTION

Recurrent pregnancy loss (RPL) represents a major health problem with two, three or more losses in up to 5% of women of reproductive age (1) and is actually one of the most common causes of female sterility. In this setting, several diseases have been identified to explain causes of RPL, such as endocrine diseases (in particular ovarian dysfunction, anovulation, hypopituitarism, diabetes), uterine malformation, genetic alterations (e.g. chromosomal aberrations), inflammatory diseases, (in particular systemic lupus erythematosus) and infectious diseases. Moreover, several reports identify inherited predisposition to thrombophilia as one of the main causes of RPL (2-4).

From a pathological point of view, women affected by thrombophilia show during their pregnancy a hypercoagulable state, which is already increased during pregnancy, which may impair placental flow and then its function and fetal growth.

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THROMBOPHILIA AND HYPERCOAGULABLE STATE IN PREGNANCY

During pregnancy we may observe many changes in the hemostatic balance with a trend toward thrombophilia in order to be prompt for the hemostatic challenge of delivery (2,5,6). Thus, pregnancy is a condition associated to thrombophilia per se, and for this reason it is associated with the increase of several clotting factors (i.e. factor VIII, vWF, fibrinogen, factor VII) (6). Moreover, also other markers of a hypercoagulable state are increased during pregnancy, such as D-dimer and/or prothrombin fragment 1+2 (7,8). For this reason we may observe an increased number of venous thromboembolic events during pregnancy (9). For this reason women carrying further thrombotic risk factors (e.g. inherited thrombophilia) show an additionally increased risk of thrombotic events during pregnancy such as venous thromboembolism (VTE) and/or abortion (10).

INHERITED THROMBOPHILIA AND PREGNANCY LOSS

In 1999 a study by Brenner et al identified thrombophilia as the main cause of more than 40% of RPL, in particular early RPL (11). Although several studies on this topic are available in the literature to confirm this trend,

rates of thrombophilia seem to differ from study to study because of different inclusion criteria and different ethnic backgrounds of the selected patients.
From a pathophysiological point of view, inherited thrombophilia may be due to deficiency of clotting inhibitors or to gene variants leading to a hypercoagulable tendency (12,13).
Deficiency of clotting inhibitors, such as protein S, protein C and/or antithrombin, has been clearly associated to RPL since 1996 in a study by Sanson et al (14). Inherited conditions frequently associated with a hypercoagulable state and RPL are prothrombin A20210G variant and/or factor V Leiden. Prothrombin A20210G has been identified as a risk factor for pregnancy loss in several studies and has been associated to early RPL but also to late RPL (15-19).
On the other hand, factor V Leiden, which is responsible for more than 75% of inherited activated protein C resistance, is the more common inherited thrombotic risk factor associated to RPL (17-22). In particular a case control study by Ridker et al. has reported an increased prevalence of FVL in women with RPL, while other studies revealed a strong relationship between FVL and early RPL (23). Moreover, FVL is also considered a risk factor for late RPL (24).

HYPERHOMOCYSTEINEMIA

A pathogenetic role of hyperhomocysteinemia (HHCY) in RPL has been suggested because HHCY is associated with thrombotic disorders, but no univocal data are available in the literature on this topic. Increasing evidence is available for the relationship between HHCY, methylenetetrahydrofolate reductase gene polymorphism C677T (MTHFR C677T) and RPL (25-29). Several reports, in fact, described an association between HHCY and MTHFR C677T and early RPL (25-29), but a different point of view seems to be offered by other authors concerning this association (30).

ACQUIRED THROMBOPHILIA

Some data available in the literature underline the role of the antiphospholipid syndrome (APS) and RPL (31-41). Although the mechanism of primary and secondary APS in the pathophysiology of RPL has not been clearly evaluated, the pathogenetic role of antiphospholipid antibodies in RPL has been recognized, because adverse pregnancy outcome is now one of the diagnostic criteria of APS according to the guidelines of the International Society of Thrombosis and Haemostasis and the American Rheumatology Association (see Table 1) (42-43). A rarer condition such as clotting factor XII deficiency has been associated with thrombotic events and RPL. In this setting, antibodies against factor XII have been frequently found and thus been associated to rare APS although a clear explanation of all involved processes is still a matter of discussion (44-46). However, also antibodies directed to other plasma proteins and/or clotting factors have been described in the literature in women affected by recurrent fetal loss and antiphospholipid syndrome (47-49), in particular against β_2-glycoprotein-I.
Interestingly, new evidence seems to be available for the role of increased maternal plasma levels of clotting factor VIII and the risk of RPL according to a study by Dossenbach et al (50).
Acquired activated protein C resistance (i.e. not associated with the presence of FVL) has been described in several women with RPL, but also in this case not all involved mechanisms are known from a pathophysiological point of view (1,51).

COMBINED THROMBOPHILIA

Combined thrombophilia (i.e. inherited thrombophilia associated with acquired thrombophilia or more than one inherited thrombophilic defect) has also been identified as a cause of RPL, but its real frequency is still a matter of discussion, although it seems to be more frequent in women with unexplained late RPL (10,52).

THROMBOPHILIA AND VTE DURING PREGNANCY

VTE is a multifactorial disease which involves acquired and inherited risk factors (12). Molecular thrombophilia, in particular inherited thrombophilia, is involved in the pathophysiology of juvenile VTE, whereas pregnancy is recognized as one of the acquired thrombotic risk factors (12). However, not all pregnant women develop VTE during pregnancy, therefore a clear evaluation of the VTE risk is suggested in any case (2). Antithrombin deficiency seems to be the inherited disorder more at risk of VTE during pregnancy, but also other molecular conditions associated with inherited thrombophilia have been frequently identified (2,9).
Thromboprophylaxis during pregnancy may be divided into primary prophylaxis or secondary prophylaxis in subjects who previously suffered from VTE. Primary thromboprophylaxis is recommended in particular for pregnant women undergoing Caesarean section and during the puerperium (2, 53, 54). Moreover, thromboprophylaxis has been also suggested for pregnant women carriers of inherited and/or acquired thrombophilia without previous history of VTE. Furthermore, asymptomatic pregnant women should undergo active thromboprophylaxis if one of these conditions is present: antithrombin deficiency, more than one thrombophilic condition (e.g. homozygosity for one thrombophilic gene polymorphism or two or more heterozygosities for thrombophilic defects), and first degree relative with a history of severe juvenile VTE (2,55,56).

Table 1: Diagnostic criteria to detect antiphospholipid syndrome

Clinical criteria

Vascular thrombosis of arterial and/or venous vessels in any tissue or organ

Pregnancy morbidity

- One or more unexplained deaths of a morphologically normal fetus at or beyond the 10th week of gestation
- One or more unexplained deaths of a morphologically normal neonate before the 34th week of gestation because of eclampsia or severe eclampsia or placental insufficiency
- Three or more unexplained consecutive spontaneous abortions before the 10th week of gestation

Laboratory criteria

- Lupus anticoagulant present in plasma, on two or more occasions at least 12 weeks apart, detected according to the International Society of Thrombosis and Haemostasis (ISTH)
- Anticardiolipin antibody (aCL) of IgG and/or IgM isotype in serum or in plasma present in medium or high titer on two or more occasions at least 12 weeks apart
- Anti-β_2 glycoprotein-I antibody of IgG and/or IgM isotype in serum or in plasma present on two or more occasions at least 12 weeks apart

For more details we suggest to consult Miyakis S, et al. J Thromb Haemost. 2006;4:295-306

In pregnant women with previous VTE secondary thromboprophylaxis during pregnancy should be considered seriously. According to the data available from the literature we may perform an active and frequent clinical surveillance because of a reported low-incidence of VTE recurrence ante-partum or an active pharmacological thromboprophylaxis based on the administration of unfractionated heparin (UFH) or low molecular weight heparin (LMWH) (57-59). However, pharmacological thromboprophylaxis is suggested if further thrombotic risk factors are present during pregnancy (i.e. prolonged bed rest, recent surgery, obesity, autoimmune disease with or without APS, infections, nephrotic syndrome, pre-eclampsia) and also during the puerperium (2).

Pharmacological thromboprophylaxis is suggested in any case during the puerperium for about six to eight weeks and based on the administration of UFH or LMWH once daily (2, 59).

Episodes of acute VTE during pregnancy should be treated as in non-pregnant women (i.e. with full doses of UFH monitored with activated partial thromboplastin time, or with LMWH twice daily) (58-62). However, many variables should be considered during heparin treatment in pregnancy because of the several metabolic changes during pregnancy that may also modify the plasma concentration and bioavailability of heparin (63). Anticoagulant treatment after acute VTE in pregnancy should be continued, in particular if needed for clinical conditions, but it should be stopped before delivery (2). After delivery anticoagulant treatment should of course be re-started and based also on oral anticoagulation according to the required INR range. (2)

THROMBOPHILIA, PREGNANCY AND HEPARIN TREATMENT

The literature offers many data concerning thromboprophylaxis based on UFH or LMWH and VTE during pregnancy, but few data are available concerning the role of heparin for the prevention of miscarriage. LMWH has been suggested to improve the pregnancy outcome in many cases because of its safety and efficacy also at low doses. However, the LIVE-ENOX study, a double-blind randomized trial, has demonstrated not only the efficacy and the safety of LMWH administered twice daily to thrombophilic pregnant women with RPL but also an improvement of pregnancy outcomes. Furthermore, this study suggested several hemostatic tests that may be performed in high-risk pregnancy during treatment with full doses of LMWH (55, 64-67).

Furthermore, the usefulness and the safety of heparin, in particular low molecular weight heparins, have been demonstrated also by several studies focusing on the prevention of abortion in women with antiphospholipid syndrome (68-70). Moreover in these studies, abortion and/or further thrombotic complications in women with APS were frequently prevented by a combined treatment based on LMWH/UFH and aspirin (69, 70)

CONCLUSION

The new evidence of a possible role of antithrombotic treatment with full doses of LMWH during high-risk pregnancy may support the idea that we may have a further chance to prevent obstetric complications also in thrombophilic women. Thus, active surveillance and an

early diagnosis of women referred to gynecological centers for this kind of reason may be helpful to fight this major health problem that involves up to 5% of women of reproductive age. However, further studies are needed to confirm this trend and also to obtain data concerning the efficacy, safety and laboratory tests to monitor this kind of treatment.

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8. M D'Uva, P Di Micco, I Strina, C Alviggi, M Iannuzzo, A Ranieri, A Mollo, G De Placido. Hyperhomocysteinemia in women with unexplained sterility or recurrent early pregnancy loss from Southern Italy: a preliminary report. Thrombosis Journal 2007; 5: 10 [original investigation]

Le alterazioni dell'emostasi sono state frequentemente associate in Letteratura alla poliabortività. Negli ultimi anni dati della Letteratura hanno anche sottolineato le alterazioni del metabolismo dell'omocisteina quale causa emergente di poliabortività sebbene non sono presenti dati univoci in Letteratura. Dati emergenti su una possibile associazione tra sterilità femminile e alterazioni dell'emostasi sono inoltre stati recentemente pubblicati da diversi Autori in donne con ripetuti insuccessi alle procedure di fecondazione assistita, mentre l'associazione tra alterazioni del metabolismo dell'omocisteina e sterilità femminile non è mai stata segnalata. Lo studio propone un'analisi dettagliata delle alterazioni del metabolismo dell'omocisteina includendo non solo la ricerca dell'iperomocisteinemia, ma anche il dosaggio dei folati plasmatici e della vitamina B12 e della variante genica C677T della MTHFR in 3 gruppi di soggetti: donne con sterilità inspiegata, donne con poliabortività, donne con normale funzione riproduttiva (gruppo controllo). Tutte le pazienti selezionate nello studio non presentavano altre associazione patologiche che potessero essere cause di sterilità o poliabortività. I dati riportati hanno confermato un'associazione tra iperomocisteinemia e poliabortività ma hanno anche sottolineato la presenza di iperomocisteinemia nelle donne sterili selezionate con valori plasmatici sovrapponibili a quelli delle donne affette da poliabortività. La medesima correlazione è stata inoltre riscontrata per la variante genica C677T della MTHFR che frequentemente si associa a iperomocisteinemia che presentava elevata frequenza sia nelle donne affette da sterilità che nelle donne affette da poliabortività. I dati riportati hanno inoltre sottolineato un'ulteriore associazione tra iperomocisteinemia, ridotti valori di folati plasmatici e sterilità femminile sinora mai segnalata nei diversi reports presenti in Letteratura.

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Hyperhomocysteinemia in women with unexplained sterility or recurrent early pregnancy loss from Southern Italy: a preliminary report

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Abstract

Background: Hyperhomocysteinemia has been described as a risk factor for unexplained recurrent pregnancy loss. Increased levels of homocysteine may be due to inadequate dietary intake of folate and vitamin B12 and inherited defects within the methionine-homocysteine pathway such as MTHFR C677T gene polymorphism. However, the association between hyperhomocysteinemia and sterility problems have been underlined only for recurrent pregnancy loss while a relationship between hyperhomocysteinemia and female sterility is still matter of discussion.

Aim: This study sought to find out a possible relationship between sterility (primary sterility or secondary sterility due to recurrent pregnancy loss) and homocysteine metabolism.

Patients and Methods: We selected 20 patients with recurrent pregnancy loss, 20 patients with unexplained female sterility and 20 healthy women as control group. Several whole blood samples were collected by venipuncture. Firstly homocysteinemia and other related variables were tested (i.e. folate and vitamin B12 levels); thereafter DNA was extracted by a further whole blood sample collected in EDTA in order to screen MTHFR C677T gene polymorphism. Statistical analysis was performed by chi square test; differences were considered to be significant if $p < 0.05$.

Results: The median fasting total plasma homocysteine concentration was 19.2 ± 6.14 μM for patients with recurrent pregnancy loss, while was 21.05 ± 8.78 μM for patients with unexplained sterility, vs 7.85 ± 3.31 μM of control group ($p < 0.05$). Fifteen patients with unexplained female sterility showed MTHFR C677T homozigosity vs 17 with recurrent pregnancy loss and 3 in the control group ($p < 0.05$). On the other hand no significant differences were found in the levels of vitamin B 12 in the three groups, while reduced folate concentrations were found in women with unexplained female sterility and recurrent pregnancy loss ($p < 0.05$ vs control group.

Discussion: MTHFR C677T gene polymorphism is frequent in the studied populations. These data raise questions on the role of the homocysteine metabolism in sterility problems. Even though increased homocysteine (i.e. > 15 μM) and MTHFR C677T homozigosity have already been described as risk factors for recurrent pregnancy loss, few studies

evaluated their role in women with unexplained sterility. Further studies on larger series are needed to better understand the role of homocysteine metabolism, including folate metabolism, in this clinical setting.

Background

Hyperhomocysteinemia (HHCY) has been underlined as an emerging risk factor for several diseases such as arterial and/or venous thrombosis [1], adverse pregnancy outcome [2,3], congenital malformations [4] and vascular dementia [5,6]. Inherited and acquired conditions have been involved to explain pathophysiology of HHCY such as gene polymorphisms [i.e. cystationin beta synthase (CBS) or methylenetetrahydrofolate reductase (MTHFR)] [7,8] and folate and/or vitamin B6/B12 deficiencies due to disregulation of their normal metabolism and/or low dietary intake [9-11]. Because HHCY has frequently been associated to clinical vascular thrombosis, homocysteine metabolism is investigated, together with other thrombophilic conditions such as inherited and/or acquired thrombophilia, in patients with early onset of vascular thrombosis [1]. However, in this field inherited and/or acquired thrombophilia are identified as well known risk factors for adverse pregnancy outcome, in particular in case of early recurrent pregnancy loss (RPL). Literature already underlined a strong relationship between inherited deficiency of protein C, protein S or antithrombin and RPL [12]. Moreover, gene variants related to thrombophilia as factor V Leiden and\or prothrombin A20210G have been reported to be associated to RPL [13-15]. Moreover, acquired conditions such as antiphopspholipid syndrome or increased plasma levels of clotting factor VIII have been associated to RPL [16-19]. Furthermore, increasing evidence is now available also for the association of HHCY and RPL [2,3].

Previous studies failed to include or exclude other causes of miscarriages, when alteration of haemostasis with a trend toward hypercoagulable state were considered. On the other hand, few emerging data are now available for the role of inherited and/or acquired thrombophilia in women with in vitro fertilisation failure [20,21], while data about the association of female sterility and alteration of haemostasis are lacking, in particular if the homocysteine metabolism is considered.

The aim of our study was therefore to investigate the role of homocysteine metabolism in patients with unexplained female sterility or secondary sterility due to RPL.

Patients and Methods

Patients selection

We observed 125 consecutive women referred to our Sterility Center for infertility due to RPL or for female sterility. We considered for this study in the group of women with RPL all patients with 2 or more first trimester abortion or with 1 or more late pregnancy loss, while we considered women without any clear evidence of pregnancy in their anamnesis in the group of women with unexplained female sterility (UFS).

In order to evaluate the causes of RPL or female sterility we looked for chromosomal alterations, endocrine dysfunctions, chronic inflammatory diseases, infectious diseases, uterine malformations, tubal patency, alteration of haemostasis with a trend toward thrombophilia.

All patients underwent karyotype study in order to detect several chromosomal aberrations such as balanced translocations.

Anatomic evaluation of the uterine cavity was performed by transvaginal ultrasound scan and hysterosalpingography and/or hysteroscopy in order to detect mullerian malformations or the presence of fibroids or polyps and\or tubal patency.

Endocrinological assessment included screening for diabetes, hypothyroidism, hypopituitarism, hyperprolactinaemia, luteal insufficiency and polycystic ovarian syndrome (PCOS). Basal FSH, LH and oestradiol, luteal phase progesterone, TSH, prolactin levels and fasting glucose were evaluated in all patients. In addition, trans-vaginal USG and androgens levels were assessed to look for PCOS and\or anovulation.

Chronic inflammations due immunological diseases such as systemic erythematosus lupus, rheumatoid arthritis, systemic sclerosis were also studied. Serum level of antinuclear antibodies (ANA), antimithocondrial antibodies (AMA), smooth muscle antibodies (SMA), rheumatoid factor and levels of C reactive protein were assessed. Infective disease due to *Chlamydia spp*, were evaluated by specific *Chlamydia* assays (i.e. serological levels of specific IgG and IgM against *Chlamydia spp*). Moreover, all patients were examined for vaginal and cervical smears, in order to exclude vaginal and\or cervical infections such as *Chlamidia spp or Mycoplasma spp* or mycosis.

Alterations of haemostasis with a trend toward thrombophilia were excluded by specific assays to test protein C, protein S and antithrombin deficiency, anticardiolipin IgG and IgM antibodies, lupus anticoagulant, inherited gene polymorphisms of factor V Leiden and A20210G

prothrombin. So, patients carrying hypercoagulable state due the reported variables were excluded from the study.

Moreover, obese patients were excluded, in particular women with Body Mass Index > 25 were not enrolled in the study.

Patients group

After this screening we selected 20 patients with unexplained RPL and 20 women with UFS.

Selected women were tested for several variables of homocysteine metabolism through methylene-tetra-hydrofolate reductase (MTHFR) C677T gene polymorphism, homocysteinemia, folate and vitamin B 12 levels. All selected patients, both affected by UFS and RPL, were taking preconceptional doses of folic acid (i.e. 400 μg) in order to prevent neural tube defects in case of pregnancy. On the other hand none of control subjects was taking folic acid.

Methods

Whole blood samples were collected from all selected subjects in the study by venipuncture from antecubital vein in order to screen possible involvement of alteration of homocysteine metabolism. All subjects were assayed for plasma homocysteine (t-Hcy), vitamin B12 and folic acid levels and MTHFR C677T gene polymorphism.

First blood sample

The first blood sample was collected in EDTA to screen fasting homocysteine (FPIA-Abbott).

Second blood sample

The second blood sample was collected in SST II advanced tube in order to detect serum folic acid levels and serum vitamin B 12 levels (CMIA-Abbott)

Third blood sample

A further blood sample (5 mL) was collected in EDTA in order to screen gene variants of MTHFR C677T. DNA was extracted using the "NUCLEON BACC" kit (Amershan, Germany). Patients were screened for the C677T gene polymorphism of MTHFR using PCR amplification with specific primers and the Light Cycler apparatus (Roche, Italy).

Control group

Twenty age and sex matched healthy subjects were enrolled as control group. Selected subjects had the same ethnical background of the study group. In the control group we included women with one or more successful pregnancy and without gestational complication (intrauterine growth restriction, stillbirth and abruptio placentae) and any abortion. We excluded subjects with previous arterial and/or venous thrombosis. We also excluded subjects with first degree relatives with arterial and/or venous thrombosis before than 65 years old.

Statistical analysis

Statistical analysis was based on chi square test, differences were considered to be significant if $p < 0.05$. Statistical analysis was carried out using SPSS statistical software [22,23].

Results

Fasting levels of homocysteinemia were higher both in patients with UFS (i.e. 21.05 ± 8.78 μM) and with RPL (19.20 ± 6.14 μM) compared to control subjects (7.85 ± 3.31 μM); differences were both statistically significant (p: <0.01) (Table 1 and 2). Fasting homocysteine was slightly increased in patients with unexplained sterility compared to patients with RPL, but this difference did not reach statistical significance (Table 1 and 2).

Moreover, serum folic acid levels were lower both in women with UFS (6.70 ± 4.50 ng/ml) and by RPL (6.10 ± 2.81 ng/ml) compared to control subjects (20.10 ± 9.44 ng/ml) and also these differences reached statistical significance (p: <0.01) (Table 1 and 2). Interestingly, in this case we found also a significant statistical difference in folate concentrations in women with unexplained sterility compared to women with RPL (p: 0.007) (Table 1 and 2).

Serum vitamin B12 levels were comparable in women with UFS (648 ± 162 pg/dl), in women with RPL (608 ± 154 pg/dl) and in control subjects (664 ± 175 pg/dl) (Table 1 and 2) not reaching significant statistical differences (Table 2).

MTHFR C677T gene polymorphism was searched for in all subjects with UFS, RPL and in controls. Five/20 patients with UFS and 3/20 patients with RPL showed heterozigosity for MTHFR C677T compared to 9/20 of control group (Table 2).

MTHFR C677T homozigosity was present in 15/20 patients with UFS and 17/20 patients with RPL compared to 3/20 subjects of control group (Table 1); differences were significant both for sterility and RPL when compared to control group (p: <0.01 UFS vs control group and RPL vs control group) (Table 2). No differences were found between UFS and RPL (p: 0.69, ns) (Table 2). None of the subjects with UFS or RPL were wild type for MTFHR C677T gene polymorphism vs 8\20 subjects of control group.

Results showing variables in patients with unexplained female sterility, RPL and control group are summarised in Table 1 and 2.

Table 1: Data of homocysteine metabolism in patients with sterility or RPL and in control subjects

Test (unit of measurement)	UFS (subjects 20)	RPL (subjects 20)	CG (subjects 20)
Hcy (μM/ml)	21.05 ± 8.78	19.20 ± 6.14	7.85 ± 3.31
Serum folic acid (ng/ml)	6.70 ± 4.50	6.10 ± 2.81	20.10 ± 9.44
Serum vitamin B 12 (pg/ml)	648 ± 162	608 ± 154	664 ± 175
MTHFR C677T heterozigosity	5/20	3/20	9/20
MTHFR C677T homozigosity	15/20	17/20	3/20

Hcy: homocysteine
MTHFR: methylenetetrahydrofolate reductase
UFS: unexplained female sterility group
RPL: recurrent pregnancy loss group
CG: control group

Discussion

Since 1990 several studies underlined a pathogenic role for inherited thrombophilia in women with RPL [24,25]. A pathogenic role was identified for inherited deficiency of protein C, protein S and antithrombin and for inherited gene polymorphism of factor V Leiden and A20210G of prothrombin, and also for acquired thrombophilia, in particular antiphospholipid syndrome [12-19].

Also a potential pathogenic role of HHCY has been recently suggested, because the association of HHCY and thrombosis, but not univocal data are available. Increasing evidences are available for the relationship between HHCY and MTHFR C677T gene polymorphism and unexplained recurrent pregnancy loss. Several reports, in fact, described an association between early RPL and HHCY and/or MTHFR C677T gene polymorphism [2,3,26,27]. A different point of view on the association between HHCY and RPL has been reported only by Makino et al. [28].

Only a few studies are available on the association between sterility and HHCY and they are focused only on women with in vitro fertilization failures. Moreover, available data seem to be in contrast: Martinelli et al. did not find an association between inherited thrombophilia and patients with in vitro fertilization failure [29], while Azem et al. and Qublan et al. underlined a possible role of inherited thrombophilia and IVF failure [20,21]. However, both studies were not based on a possible association of homocysteine metabolism and IVF failure but only on the C677T gene polymorphism of MTHFR.

In the present study we evaluated not only homocysteinemia and MTHFR C677T gene polymorphism but also other common variables associated with homocysteine metabolism such as folate and vitamin B12 serum levels both in women showing RPL and women with UFS.

We found that women with RPL and UFS showed HHCY compared to control group (Table 1 and 2). We did not find differences in homocysteine levels between women with RPL and women with UFS. Although data concerning HHCY and RPL seem to be in agreement with those already reported [2,3,26,27], data focusing the association between of HHCY and UFS are innovative because rarely described so far.

Data concerning MTHFR C677T gene polymorphism seem to support this hypothesis because we found a high frequency of homozigosity of TT genotype not only in

Table 2: Statistical differences between patients with unexplained female sterility, patients with RPL and control subjects

	Hcy (μM/ml)	p	Serum folate (ng/ml)	P	Serum vit. B 12 (pg/ml)	p	MTHFR C677T heterozigosity	p	MTHFR C677T homozigosity	p
UFS vs CG	21.05 ± 8.78 vs 7.85 ± 3.31	<0.01, s	6.70 ± 4.50 vs 20.10 ± 9.44	<0.01, s	648 ± 162 vs 664 ± 175	0.35, ns	5/20 vs 9/20	0.32, ns	15/20 vs 3/20	<0.01, s
UFS vs RPL	21.05 ± 8.78 vs 19.20 ± 6.14	0.44, ns	6.70 ± 4.50 vs 6.10 ± 2.81	0.007, s	648 ± 162 vs 608 ± 154	0.42, ns	5/20 vs 3/20	0.69, ns	15/20 vs 17/20	0.69, ns
RPL vs CG	19.20 ± 6.14 vs 7.85 ± 3.31	<0.01, s	6.10 ± 2.81 vs 20.10 ± 9.44	<0.01, s	608 ± 154 vs vs 664 ± 175	0.20, ns	3/20 vs 9/20	0.08, ns	17/20 vs 3/20	<0.01, s

Hcy: homocysteine
MTHFR: methylenetetrahydrofolate reductase
UFS: unexplained female sterility group
RPL: recurrent pregnancy loss group
CG: control group
ns: not significant
s: significant

women with RPL but also in women with UFS (Table 1). TT genotype of MTHFR C677T gene polymorphism has been already underlined in pathophysiology of RPL by several Authors but its role in pathophysiology of UFS is still a matter of discussion. Therefore, our data support the hypothesis concerning the involvement of homocysteine metabolism in cases of UFS because frequency of TT genotype was similar to that observed in women with RPL (Table 2). Moreover, our data may also support previous observations of an increased frequency of thrombophilia in women with infertility showing repeated IVF failures.

Serum folate and vitamin B12 levels were also tested to support this hypothesis because strongly associated to homocysteine metabolism, also in terms of a possible therapeutic support. Although, vitamin B12 levels were lower both in women with RPL and with UFS compared to control subjects, and these differences did not reach statistical significance (Table 1 and 2). On the other hand we found significantly lower folate levels both in women with RPL and UFS when compared with control subjects (Table 1 and 2). These data confirm the strict association between folate metabolism and homocysteine levels also in pathophysiology of women with infertility, in particular if referred to adverse pregnancy outcome such as early RPL. Moreover, for the first time we underlined a possible association between low serum folate and HHCY and UFS. Furthermore, from another point of view our data offer a new scenario on the possible therapeutic support with folic acid fortification both in women with RPL and UFS carrying HHCY.

In conclusion our study provides several data concerning the involvement of homocysteine metabolism in women with infertility: we confirmed a strict association between HHCY and TT genotype of MTHFR C677T in women with RPL without other causes of recurrent abortion. Yet, for the first time, we suggested also that homocysteine metabolism may be involved in pathophysiology of these cases of UFS because of the association between HHCY, low serum folate and TT genotype of MTHFR C677T. However, because of the small number of selected patients, our data should be confirmed by further studies based on larger population.

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Parte 5 - Discussione.

Le patologie della riproduzione femminile rappresentano un rilevante problema della Sanità soprattutto nei Paesi Occidentali arrivando a interessare sino al 5-10% di donne in età riproduttiva potenzialmente affette da sterilità o poliabortività (96). Negli ultimi decenni particolare attenzione è stata rivolta all'associazione tra poliabortività e alterazioni coagulative con riferimento agli stati di ipercoagulabilità. I risultati delle diverse casistiche disponibili relative all'incidenza delle trombofilie congenite, acquisite o combinate nelle popolazioni studiate non appaiono omogenei, probabilmente a causa dei differenti criteri di inclusione e/o di esclusione adottati (69, 70, 72, 77, 78, 79, 80, 81, 82, 85, 87, 88, 89). Un'ulteriore variabilità dei risultati potrebbe essere legata alle diverse etnie delle donne selezionate nei vari studi.

L'obiettivo di questo lavoro di tesi di dottorato è stato quello di identificare eventuali associazioni tra trattamenti ormonali ed eventi trombotici del versante arterioso o venoso verificatisi in qualsiasi organo o distretto e di ricercare il ruolo delle alterazioni dell'emostasi in donne affette da sterilità idiopatica o da poliabortività inspiegata.

Nella nostra esperienza clinica abbiamo riscontrato un evento di trombosi carotidea con stroke ischemico in una donna sottoposta a cicli ripetuti di iperstimolazione ovarica controllata **(R1)**, e una trombosi della vena mesenterica superiore in una donna in trattamento con estro-progestinici a scopo anticoncezionale **(R6)**. In entrambi i casi le pazienti non presentavano stati di trombofilia congenita o acquisita dipendente da alterazioni molecolari, suggerendo che in taluni casi le patologie trombotiche possano dipendere dai soli fattori di rischio ambientali-iatrogeni e indurre complicanze vascolari temibili.

Recenti casistiche riportano inoltre scarsa associazione tra trombosi venose del distretto ovarico e trombofilie congenite o acquisite legate ad alterazioni molecolari (97). Tali distretti venosi sono sedi atipiche di trombosi venosa profonda e molta attenzione deve essere posta alle condizioni acquisite di rischio trombotico tra le quali il puerperio, le sovrainfezioni distrettuali e gli

approcci chirurgici loco-regionali. Tuttavia, la difficoltà nella gestione delle trombosi venose ovariche sembra essere legata principalmente ai tempi diagnostici e alle procedure di imaging necessarie per porre una diagnosi di certezza **(R5)**.

L'associazione tra diversi stati trombofilici di natura congenita, di natura acquisita, di natura combinata e poliabortività è già nota da diversi anni e anche nella nostra esperienza clinica quotidiana discretamente rappresentata nelle donne affette da poliabortività **(R7)**. Numerose casistiche disponibili in Letteratura confermano in queste pazienti un ruolo rilevante delle trombofilie congenite, in particolare il polimorfismo del fattore V Leiden, la variante genica A20210G della protrombina e l'iperomocisteinemia associata o meno alla variante genica C677T della MTHFR (69, 70, 77, 78, 79, 80, 81, 88, 89); tra le trombofilie acquisite un aspetto preponderante è rappresentato dalla sindrome da anticorpi antifosfolipidi sia primitiva che secondaria (72, 85). Le sindromi antifosfolipidi primitive, ad eziologia tuttora ignota, possono inoltre avere numerose varianti sia cliniche che laboratoristiche; in un caso da noi descritto un anticorpo atipico, diretto contro il fattore coagulativo XII, è stato identificato e considerato causa delle alterazioni biochimiche presenti nella paziente quali prolungamento dell'aPTT, riduzione dei livelli di fattore XII, positività transitoria al LAC **(R4)**.

I dati presenti in Letteratura hanno comunque dimostrato che le donne affette da poliabortività e che presentano alterazioni della coagulazione con *trend* all'ipercoagulabilità si giovano di trattamenti antitrombotici che sono in grado di migliorare il loro *outcome* riproduttivo. I diversi studi hanno anche evidenziato che le eparine a basso molecolare sono tra i farmaci antitrombotici più sicuri sia da un punto di vista della tollerabilità che del miglioramento dell'outcome riproduttivo, in particolare l'enoxaparina utilizzata alla dose di 4000UI sq die o 4000UI sq bid (90). In tali studi inoltre l'utilizzo dell'enoxaparina ha dimostrato affidabilità anche nel monitoraggio laboratoristico di alcuni marcatori della ipercoagulabilità **(R3)**. Tra questi marcatori la nostra esperienza clinica ha dimostrato l'importanza anche in fase diagnostica, del dosaggio del d-dimero nell'identificazione delle donne

potenzialmente portatrici di trombofilia congenita e\o acquisita. Livelli elevati di d-dimero sono stati riscontrati in molte pazienti da noi osservate sia per poliabortività che per sterilità e l'80% di queste è successivamente risultata positiva a screening specifici per trombofilia congenita e\o acquisita **(R2)**.

In donne affette da sterilità inspiegata lo studio del metabolismo dell'omocisteina ha dato risultati molto incoraggianti per i possibili sviluppi futuri nell'esperienza clinica delle alterazioni dell'emostasi in Ginecologia. Infatti in una rilevante percentuale di pazienti affette da sterilità inspiegata e poliabortività, e per le quali tutte le altre potenziali cause patologiche erano state precedentemente escluse, abbiamo riscontrato un'alterazione del metabolismo dell'omocisteina con *trend* all'iperomocisteinemia **(R8)**.

L'iperomocisteinemia potrebbe quindi essere responsabile di alcune forme di sterilità oltre che di poliabortività e le implicazioni scientifiche e cliniche delle osservazioni riportate, data la ridotta presenza di dati disponibili in Letteratura, potrebbero essere rilevanti sia da un punto di vista diagnostico che terapeutico.

In sintesi dai risultati di questo lavoro di tesi di dottorato si potrebbe ipotizzare un protocollo di screening delle alterazioni dell'emostasi anche in donne affette da sterilità idiopatica oltre che in quelle poliabortive.

Inoltre, allo scopo di migliorare l'outcome riproduttivo di pazienti che presentino alterazioni della coagulazione con *trend* all'ipercoagulabilità, si potrebbero ipotizzare *flow-chart* associate ad attenti monitoraggi clinico-laboratoristici associate all'utilizzo di farmaci antitrombotici potrebbe essere un nuovo punto di partenza per lo sviluppo di protocolli terapeutici sicuri.

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Tabelle.

Tabella 1. Le principali cause di trombofilia ereditaria.

- deficit di antitrombina III
- deficit di proteina C
- deficit di proteina S
- resistenza alla proteina C attivata
- fattore V Leiden
- fattore II G20210A
- iperomocisteinemia

Tabella 2. I fattori di rischio per trombosi venosa profonda.

- immobilizzazione
- stato post operatorio (ortopedia, ginecologia, chirurgia addominale maggiore)
- traumi
- età avanzata
- tumori maligni
- insufficienza cardiaca avanzata
- insufficienza venosa cronica
- gravidanza/puerperio
- contraccettivi orali
- obesità
- sindrome nefrosica
- sindrome da anticorpi antifosfolipidi (LAC)
- trombofilie ereditarie