



**STRATEGIC COMMITTEE AND STUDY GROUP ON IMMUNODEFICIENCIES
ITALIAN ASSOCIATION OF PAEDIATRIC HAEMATOLOGY AND ONCOLOGY**

**ATAXIA TELANGIECTASIA
Recommendations for diagnosis and treatment**

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AIMS

The recommendations on the management of patients with Ataxia Telangiectasia (A-T) are part of a policy to formulate recommendations for the diagnosis and treatment of rare diseases, already devised for patients with chromosome 22 deletion. Although only a single genetic defect is involved, Ataxia Telangiectasia is a polysystemic disease, i.e. affecting many parts of the body (neurological, immunological, endocrinological, etc.). Ataxia Telangiectasia is caused by mutations in the *ATM* gene (Ataxia-Telangiectasia Mutated) resulting in a total or subtotal absence of the ATM protein known to play a key role in regulating the cell cycle. Given its polysystemic nature, appropriate care of patients with A-T requires a team of specialists working together.

The basic aims of these recommendations are to:

- guarantee standard nationwide care;
- monitor the natural history of the syndrome in relation to variations in clinical presentation;
- improve the quality of life of A-T patients;
- Collect a case series sharing the same underlying genetic defect.

The first part of these diagnostic and therapeutic recommendations addresses the pathophysiological mechanisms underlying the clinical and phenotypic spectrum of Ataxia Telangiectasia.

The second part describes the diagnostic protocol, indicating patients' inclusion criteria and the specialists involved in a full patient work-up.

The third part outlines the guidelines for the multidisciplinary management of A-T patients.

The fourth part deals with the problems of genetic risk, including screening for carrier status in families at risk, and prenatal diagnosis.

1. INTRODUCTION

Ataxia Telangiectasia (A-T) is a multisystem disease inherited in an autosomal recessive fashion and characterized by cerebellar ataxia, telangiectasia in the skin and eyes, immunodeficiency, radiosensitivity and an increased incidence of tumours both in A-T patients and their relatives (Boder, 1985). A-T cells are hypersensitive to ionizing radiation and radiomimetic substances due to failure to activate the cell cycle checkpoints after treatment with these agents (Taylor et al., 1975). The hypersensitivity to ionizing radiation was disclosed for the first time in an A-T patient exposed to radiotherapy at conventional doses that proved fatal (Gotoff, 1967). The gene responsible for the disease, *ATM* (A-T mutated), belongs to a family of genes well preserved on the evolutionary scale that regulates the cell cycle checkpoints and programmed cell death (Savitsky et al., 1995; Delia et al., 2003; Shiloh, 2003). Cerebellar ataxia first appears around the first year of life at the toddler stage with frequent falls and a wide-based ataxic gait. A characteristic truncal ataxia may already be apparent at six months of age with anteroposterior and lateral oscillations. The ataxia is progressive and most A-T patients will be using a wheelchair by puberty. It is caused by a degeneration of Purkinje cells which appear reduced in number and markedly pycnotic at cytological examination. In rare cases children do not develop ataxia until puberty or present choreoathetosis at onset. The other neurological hallmark of A-T is oculomotor apraxia varying in onset but present in almost all patients after two years of age.

Other common neurological signs encountered in A-T patients include restricted eye movements (strabismus, nystagmus) and slurred speech (dysarthria) (progressively worsening to the point that patients can no longer speak coherently, giving the impression of being mentally retarded, whereas this is very rare in A-T), abnormally low muscle tone (hypotonus) and sometimes epilepsy. Prominent blood vessels (telangiectasias) are the second hallmark of the disease, appearing in most A-T patients between two and eight years of age (average age 72 months) (Harding 1988). Immune problems varying in severity are present in around 60% of A-T patients and involve both humoral and cell-mediated immune responses (Chun and Gatti, 2004; Gatti, 1982; Gatti, 1991). Around 30% of A-T patients do not have immunodeficiency so that the absence of immune problems should not preclude a diagnosis of Ataxia Telangiectasia.

The clinical diagnosis of Ataxia Telangiectasia may be difficult before the characteristic skin and eye telangiectasias become evident. Discovery of the *ATM* gene, whose mutations are responsible for the disease, has paved the way to more accurate laboratory diagnosis. The classic A-T phenotype is caused by mutations in both alleles of the gene that truncate or destabilize the protein product. The *ATM* gene is located on chromosome 11q22.3 and encodes a protein that plays a key role in regulating the cell cycle and in repairing double-stranded DNA (Savitsky et al., 1995; Delia et al., 2003; Shiloh, 2003). Molecular tests will readily distinguish the clinical A-T phenotype from that of other recessive cerebellar ataxias like Friedreich's ataxia, Ataxia telangiectasia-like disorder, oculomotor apraxia types 1 and 2, and Nijmegen breakage syndrome. Conversely, some atypical patients with mild or minimal signs of disease (late onset mild slowly progressive ataxia or late onset spinal muscular atrophy) may now be classified as A-T on the basis of absent ATM protein or mutations in the encoding gene (Chun and Gatti, 2004) (see Table 1).

Table 1 – Clinical and cellular signs of A-T, AT-LD, NBS, AOA1, AOA2

Disease	A-T	AT-LD	NBS	AOA1	AOA2
Mutated gene	<i>ATM</i>	<i>Mre11</i>	<i>NBS1</i>	<i>APTX</i>	<i>SETX</i>
Chromosome	11q23	11q21	8q21	9q13	9p34
<i>Clinical phenotype</i>					
Age at onset	2-6 years	2-6 years	1-4 years	2-6 years	11-22 years
Progressive cerebellar ataxia	+	+	-	+	+
Oculomotor apraxia	+	+	-	+	+
CNS abnormalities	-	-	+	-	-
Neuronal degeneration	+	+	-	+	?
Predisposition to cancer	+	-	+	-	-
Immunodeficiency	+	-	+	-	-
Telangiectasia	+	-	-	-	-
Elevated alpha-fetoprotein	+	-	-	-	+
Hypoalbuminaemia	-	-	-	+	-
Hypercholesterolaemia	-	-	-	+	-
<i>Cell phenotype</i>					
Radiosensitivity	+	+	+	-	-
Chromosome fragility	+	+	+	-	-
Radioresistant DNA synthesis	+	+	+	-	-

1.1 Epidemiology

The disease is found in all populations at varying frequency depending on the different rates of consanguinity and the clinical capacity to differentiate A-T from similar diseases. The incidence in the USA population is estimated at one in 40,000 live births (Swift et al., 1986). The carrier rate is between 0.5 and 2.0% among the general population. On the basis of consanguinity and applying Dahlberg's formula, an epidemiological survey of 72 Italian A-T families registered with the Italian Registry for Ataxia (RIAT) extrapolated a theoretical disease frequency of one in every 7090 conceptions and a healthy carrier rate from 1.69 to 3.43% of the population (Chessa et al., 1994). The average survival of A-T patients is 19 – 25 years, with a wide range of variability (Crawford 2006). Life expectancy is irrespective of the severity of neurological manifestations.

Death is caused by recurrent bronchopulmonary infections associated with a state of cachexia, or cancer in 10-15% of cases. The life expectancy of A-T patients has improved radically in the last 20 years due to the general improvement in living conditions and treatments, and to the identification of patients with "variant" forms of disease. Nowadays many patients live to the age of 25 years and some even survive until 40 or 50 years (Doerk et al., 2004). Respiratory insufficiency, with or without identifiable infections, is the major cause of morbidity and mortality in long-term A-T survivors.

1.2 Genetics and pathophysiology of A-T

The mutated gene in patients with Ataxia Telangiectasia was mapped in 1988 on the long arm of chromosome 11 in the 11q22-23 region (Gatti et al., 1988). However, it was only thanks to the participation of many groups in an international consortium in 1995 that the gene region was restricted to the 500kb interval (Foroud et al., 1991; Gatti et al., 1994; Lange et al., 1995) and the gene identified by positional cloning and called *ATM* (Ataxia-Telangiectasia Mutated) (Savitsky et al., 1995). *ATM* is a very large gene extending for more than 150 kilobases of genomic DNA and comprising 66 exons, 62 of which are encoding. The transcript consists of 9168 nucleotides and encodes for the ATM protein containing 3056 aminoacids and belonging to the family of phosphatidylinositol 3-kinases (PI3-K). The ATM protein plays a key role in controlling the cell cycle and repairing DNA double-strand breaks (Lavin and Shiloh, 1997; Shiloh, 2003). ATM acts by recognizing and facilitating the repair of a subcategory of double-strand breaks (DSBs) or a form of damage like oxidative stress that is converted into DSB in the DNA. Recognition probably leads to the activation of cell cycle checkpoints. ATM is present in the nucleus as an inactive dimer and is activated in response to DSBs due to autophosphorylation of serin 1981 that causes dimer dissociation in the active monomer form able to phosphorylate many target proteins (Bakkenist and Kastan, 2003). Errors in the repair of DSBs, the most lethal form of DNA damage, give rise to small deletions or insertions at the lesion site that may result in chromosomal translocations and genomic instability and finally cancer. Progressive neurodegeneration could be the outcome of a defect in controlling the cell cycle in post-mitotic cells (Yang and Herrup, 2005), a theory also supported by evidence of a mitotic checkpoint defect in A-T cells after irradiation (Takagi et al., 1998).

From the mutational standpoint, patients are usually compound heterozygous for two distinct mutations inherited from their parents, but patients homozygous for the same mutation are not uncommon, especially among populations with a high rate of consanguinity as in Italy. More than 400 different mutations have been identified to date spread throughout the *ATM* gene with no evidence of mutational hot spots (<http://chromium.liacs.nl/lovd/>). Most mutations give rise to a highly unstable truncated protein that produces a null phenotype in around 85% of cases. In many countries, including Italy, recurrent mutations have been identified whose carriers share a common haplotype indicating a founder effect (Gilad et al., 1996; Ejima and Sasaki, 1998; Laake et al., 1998; Stankovic et al., 1998; Telatar et al., 1998). The haplotype serves to identify carriers, especially when the mutation has not been identified.

Mutations have been identified in more than 90% of Italian patients. Most mutations are truncated as shown by the absence of the ATM protein in patients' cells. In addition, recurrent mutations have been identified the commonest being mutation 7517 del(4) in exon 53 found in patients with classic A-T and A-T variants.

1.3 Clinical and immunological phenotype

1.3.1 Neurological manifestations

A-T is a neurodegenerative disease characterized by the onset and slow progression of different neurological deficits. Phenotypic variability is largely the result of the timing of emergence of different symptoms and the speed of disease progression as the key symptoms of the illness are usually all present in adult patients (Chun and Gatti, 2004).

Cerebellar ataxia is the earliest and most prominent neurological symptoms of A-T and almost always the reason for the first specialist consultation.

Early clinical signs. Most children with A-T appear normal at birth and have the same psychomotor development as their peers until the time of walking. However, disease onset is associated with a prolonged phase of postural uncertainty with frequent truncal oscillations and falls often noted by parents as the first cause for alarm in an otherwise normal child. On close questioning the parents will recall the early walking stage noting delayed postural motor development, described as clumsiness, decreased muscle tone or instability while sitting. Although they do not resolve, these symptoms may sometimes appear to improve between three and seven years of age, delaying the first specialist consultation. In other cases, symptoms persist leading the parents or paediatrician to make a neurological referral which at this stage may often prove inconclusive or misleading from a diagnostic standpoint (Chun and Gatti, 2004; Perlman et al., 2003; Miller, 2004). This is because the early symptoms of truncal ataxia in the first year of life are sometimes construed as excessive instability of the trunk and head while sitting and standing, proximal hypotonia, slowness in using the arms and handling objects (Leuzzi et al., 1993; Sedgwick and Boder, 1972). These signs may be extremely subtle and in some cases not present at such an early age.

Walking and posture. Frank cerebellar ataxia is usually noted at the toddler age between 16 and 18 months. Despite broad interindividual variations, the ataxia presents hallmarks highly suggestive of A-T. Walking is unsteady primarily because of a lack of synergy between trunk and limbs already evident on standing. Slow truncal oscillations require ongoing adaptations of the limbs or trigger other compensation mechanisms with frequent adjustments of the head position. Walking tends to involve sudden uncoordinated movements: half of the body is projected forwards while the corresponding limb is swung out in an adduction movement, crossing the walking line and excessively reducing the support base at the time of load-bearing. Irrespective of the actual ataxia, bradykinesia and hypokinesia are typical features of A-T ataxia. Despite these difficulties, A-T children are able to adopt a strategy to compensate and correct their unsteady walking for a long time. Fast walking is one way of compensating because less balance is needed for quick movements (Crawford, 1998; Crawford et al., 2000). As time passes, the unexpected amplitude of each step is exacerbated by the irregular walking pace. Some patients will present dystonic foot postures (equino-varus-supinated) balancing on the forefoot in the load-bearing phase. Lastly, worsening bradykinesia and hypokinesia added to restricted eye movements linked to the onset of ocular dyspraxia (see below) make walking increasingly strenuous and ultimately no longer possible.

Unsteady posture also presents characteristic features in A-T. The trunk swings from one extreme to the other, often to the point of losing control of body posture. Patients will sometimes adopt awkward unnatural postures before a corrective reaction occurs which in turn is excessive and a cause of further destabilization. With time and disease progression many patients present rapid segmental or focal movements of the trunk in the form of adventitious postural-induced jerks, but they may also attempt late uncoordinated postural adjustments. Head instability shares the same features, often with prolonged changes in position from one extreme to the other, in extreme flexion or extension before a

correction or even overcorrection. Head movements are influenced early on by the appearance of ocular dyspraxia (see below).

From two years of age other signs of cerebellar dysfunction appear including dysarthria, loss of facial expression with reduced muscle tone and salivation, and a generalized decrease of muscle tone.

The ataxia progresses slowly but inexorably and by 12 years of age most children with AT are unable to walk unaided (Perlman et al., 2003). Whereas this event occurs with some degree of interindividual variability, the progressive worsening of motor coordination is apparent from the first years of life.

Ataxia of the upper limbs, intentional tremor and segmental myoclonus appear later making drawing and writing difficult and then impossible after the age of ten.

Brain MRI is often normal in the first two years of life but subsequently (but not invariably) discloses signs suggestive of cerebellar atrophy.

Extrapyramidal signs. Involuntary movements are common in A-T. Choreoathetosis is present in around 90% of patients and sometimes prevails over the signs of ataxia. Segmental myoclonus may affect both the extremities and the trunk (Miller 2004). From the first years of life children present rapid choreiform movements of the hands and feet in performing intentional tasks hands which often go unrecognized. Later still, intentional movements of the arms become imprecise and uncoordinated as a result of cerebellar dysmetria, and ballistic movements also become ataxic with unsteady posture and action tremor.

Dystonia (in the form of progressive athetosis of the fingers) is commonly encountered in children and adolescents, while early torsion dystonia involving the head and limbs has been reported in some cases with involvement of the proximal muscles with a flexed trunk posture developing as the dystonia worsens (Bodensteiner et al., 1980).

During the second decade of life the movement disorders worsen and severe postural tremor, intentional tremor, dysmetria, multifocal myoclonus, tic-like movements and chorea are common.

Bulbar functions and facial expression. All A-T patients have difficulty speaking (Crawford 1998). Most A-T children are dysarthric and dysprosodic. After the age of five years the dysarthria tends to worsen and speech becomes slow and words articulated with the emphasis typical of cerebellar dysarthrias involving motility of the lips, tongue and palate. Another later but constant findings is difficulty chewing and swallowing, responsible for "ab ingestis", often secondary, bronchopulmonary infections. The time required to feed increases markedly as the disease progresses and inadvertent aspiration of food becomes dramatically more important in the second decade of life. Increasing difficulty feeding is accompanied by weight loss and slow height gain.

After the first years of life, the facial expression of A-T children diminishes and at school age their mask-like expression may mimic mental retardation which is usually absent. This impression is however dispelled by a broad pleasing smile or wink, but these too subside during the second decade of life becoming less expressive.

Oculomotor deficits. Due to their early onset, the oculomotor deficits of A-T are particularly suggestive and reflect involvement of cerebellar and extrapyramidal functions caused by the functional impairment of the flocculus (Crawford, 1998). They are present in virtually

all A-T patients and often herald the onset of telangiectasia, thereby constituting an important diagnostic sign. With head fixed, intentional oculomotor movements start after a prolonged latency and are continuous, but unlike ophthalmoplegia, will be completed if a sufficiently long time is allowed. When the head is turned rapidly towards a peripheral stimulus the eyes deviate tonically in the opposite direction then slowly follow the direction of the head. Ocular movements are smooth and normal in amplitude when the head is mobilized passively, whereas the impaired conjugate motility of the eyes mainly apparent with intentional movements. Optokinetic nystagmus is absent. Often evident in the early years of life, strabismus tends to disappear. The oculomotor disorder in A-T impairs the neural mechanisms responsible for stabilizing the image on the retina and hence implicated in maintaining and inhibiting fixation. Gaze inhibition in particular is a prerequisite for implementing a change in gaze (Lewis et al 1999; Miller 2004). The progression of these deficits makes reading and writing increasingly difficult and ultimately no longer possible.

Peripheral neuropathy and myelopathy. Whereas muscle strength and osteotendinous reflexes are normal in A-T children in the first decade of life, signs of peripheral neuropathy will appear at around the age of ten years (disappearance of O-T reflexes, loss of vibratory sensitivity and leg position sense). However a frank Romberg sign is never observed. Neurophysiopathological and neuropathological studies have disclosed demyelination and astrocyte proliferation of the dorsal vertebrae and degeneration of the anterior horn neurons. Peripheral nerve studies have demonstrated a primarily axonal polyneuropathy (Stankovic et al., 1998). However, the clawfoot or horsefoot encountered in A-T children often has a dystonic rather than peripheral origin. Although it is similar to the clawfoot seen in other neuropathies (Friedreich's ataxia, Charcot-Marie-Tooth, etc.), the foot deformity in A-T is often accentuated with walking, is not associated with a rigid arcus plantaris, forefoot atrophy or hallux flexus, and is often encountered early on when the O-T reflexes are not yet evoked.

A significant number of older A-T patients develop a neurogenic amyotrophy caused by atrophy of the anterior horn motorneurons and dorsal nerve root ganglia of the spinal cord. The signs of atrophy mainly affect the hands and feet.

Cognitive development. Cognitive functions are also spared in adulthood although they may be difficult to assess due to the severe dysarthria (Gatti et al., 1991; Miller, 2004). Most A-T patients have an IQ within or slightly above normal range, while no cases of IQ below 50 have been reported (Gatti et al., 1991).

There is no evidence of cognitive deterioration in the first decade of life, and development appears to stabilize. In general verbal IQ in adult A-T patients is lower than in normal subjects (Mostofsky et al., 2000). A short-term memory deficit has been reported in the few older A-T patients in the third decade of life (Gatti et al., 1991).

1.3.2 Cutaneous manifestations

Telangiectasias are the second hallmark of A-T and occur in most but not all patients (*ataxia sine telangiectasia*). Telangiectasias consist in prominent blood vessels in the whites of the eyes, occasionally present at birth yet in other A-T patients may not develop until the teenage years. Telangiectasias typically appear in the conjunctiva where they start in the conjunctival fornix and then increase to cover the entire conjunctiva. Other areas

typically affected include the flexor regions of the limbs and neck, the retroauricular region, eyelids and more seldom other body regions. Telangiectasias may be absent in some rare cases (Willems et al., 1993). There is anecdotal evidence of angiectasias arising in the wall of the bladder or in the lungs after radiotherapy or treatment with chemotherapeutic agents like vincristine (Chun and Gatti, 2004).

Other characteristic cutaneous features are progeric changes including early grey hair, reduced skin elasticity, poikilodermy, café-au-lait spots, hypertrichosis and senile keratosis (Cohen, 1984). Acanthosis nigricans and eczema may also appear (Cohen et al., 1984). All these manifestations progressively increase with age. Skin granulomas may appear in rare cases similar to those more commonly encountered in common variable immunodeficiency (CVID) (Mitra, 2005).

1.3.3 Immunological manifestations

Around 60% of A-T patients present varying degrees of immune deficiency, while 30% do not have immune problems (Woods et al., 1992). Hence the lack of an immune deficit does not preclude a diagnosis of A-T. The immune deficit in A-T consists in impaired antibody and T-cell responses (Gatti, 1991).

The impaired antibody responses are associated with a reduced number of B cells and abnormal immunoglobulins. Around two thirds of patients present a marked reduction or absence of IgA and IgE, commonly associated with a deficit of subclasses IgG2 and IgG4. Some patients have a serum immunoglobulin profile compatible with a hyper-IgM phenotype. Unlike the idiopathic IgA deficit, A-T patients seldom have allergic reactions to the IgA contained in human gammaglobin preparations for i.m. or i.v. use. This is probably due to the frequent concurrence of IgE deficit in A-T patients as opposed to the high incidence of IgE-mediated manifestations in idiopathic IgA deficit.

Oligoclonal immunoglobulins accompanying episodes of lymphocyte proliferation (lymphadenopathy, hepatosplenomegaly) are commonly found in A-T patients.

A broad range of T-cell abnormalities are present in A-T (Fiorilli, 1982) including lymphopenia, a reduced response to mitogens and impaired T-cytokine production (Paganelli, 1984). The T-cell defect may be due to a reduced thymopoiesis resulting in the generation of a limited T-cell reservoir (Giovannetti, 2002). Determination of lymphocyte subpopulations shows a marked decrease of subpopulations CD3⁺ and CD4⁺ with normal or slightly raised values of CD8⁺. The CD4⁺/CD8⁺ ratio is often inverted. There is also a depletion of D45RA⁺ lymphocytes, so-called T-virgin cells, preferentially localized in the lymph nodes. There is often a relative increase in TCR γ/δ -bearing with respect α/β -bearing T cells or those with compound hybrid TCR genes, i.e. part γ and part β , 50-100 times more common than in normal subjects. As a result there is a reduced generation of $\alpha\beta$ T cells (Carbonari, 1990) whereas individual T cell function is spared (Pashankar, 2006). The clinical manifestations of immune deficiency in A-T are similar to those characteristic of antibody defects: recurrent infections of the upper and lower airways and gastrointestinal infections/infestations. Encapsulated bacteria are the most commonly isolated, mainly *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*. There is a markedly impaired response to pneumococcal polysaccharides (Sanal, 1999), but combined immunization with the PCV7 and PPV23 vaccines appears to trigger a significant anti-pneumococcal response in A-T patients (Stray-Pedersen, 2005). Although many patients present a cell-mediated immune defect, viral and fungal infections, pneumocystis and intracellular bacterial infections are uncommon in A-T

(Nowak-Wegrzy, 2004). In addition, vaccinations with attenuated viruses do not appear to give rise to complications in A-T patients (Nowak-Wegrzy, 2004).

Infections remain one of the main causes of death in A-T despite prompt aggressive treatments. As in patients with agammaglobulinaemia or cystic fibrosis, the chronic evolution and recurrent respiratory infections frequently give rise to pulmonary fibrosis and respiratory insufficiency. Although the severity of the neurological defect is not correlated to overall survival (Crawford, 2006), the neurological problems limit the possibility of delaying lung damage by respiratory physiotherapy and facilitate respiratory infections from aspiration and stagnation.

1.3.4 Other clinical manifestations

More than half of A-T patients have glucose intolerance, insulin resistance and hyperglycaemia. Most patients present a somatic hypoevolutism: height and weight are usually below the third percentile in adolescence, mainly in patients with recurrent or chronic respiratory infections. No hormone deficits responsible for delayed body growth have been found. The absence or delay in pubertal body changes may be associated with atrophy of the gonads and later insulin-resistant diabetes (Crawford, 1998).

Female A-T patients often have a late start of menstruation and delayed development of secondary sexual characteristics. The ovaries are sometimes absent or hypoplastic and patients are therefore sterile. By contrast, spermatogenesis and ejaculation were reported as normal in the few male A-T patients examined, and sexual reproduction has been noted in the rare A-T subjects surviving to adulthood (Stankovic et al., 1998).

Around half of A-T patients have elevated levels of liver enzymes like alkaline phosphatase and transaminases. Fatty infiltration of the portal liver cells has been described in some biopsies and is an occasional finding of little clinical significance. Variable levels of biological markers of the disease have also been noted (ATM protein levels, serum alpha-fetoprotein, radiosensitivity (see Chun and Gatti, 2004; see below - *diagnosis*).

1.3.5 Cancer risk and radiosensitivity

A-T patients have an increased incidence of tumours which are the second commonest cause of death. One in three A-T individuals will develop a tumour in the course of their lives and cancer may occasionally arise even before the diagnosis of A-T is suspected. Tumours occurring before the age of 20 years are leukaemia or lymphomas (namely T-cell lymphomas) in 85% of cases, with an increased incidence of 70 and 250 fold respectively. Acute T-cell leukaemia and T-cell lymphomas are more common in childhood, while T-cell prolymphocytic leukaemia (T-PLL) accounts for 10% of T-cell tumours thereafter. B-cell tumours encountered in older A-T patients are leukaemias and lymphomas which have no cytogenetic or clinical specificity and are present in the same tumours in the general population. After the age of 20 years solid (mainly epithelial) tumours are more common and include dysgerminomas, brain tumours (astrocytomas, medulloblastomas and gliomas), gastric, pancreatic and hepatic carcinomas, and retinoblastoma. Cases of rhinopharyngeal carcinoma have also been reported. The frequency of these tumours is slightly higher than in the general population, whereas solid gynaecological, ovarian and

uterine tumours are more common in A-T patients than in women of the same age. All these tumours should be treated with attenuated protocols (see chemotherapy).

Some A-T patients with cancer treated with radiotherapy presented an acute and chronic reaction of irradiated healthy tissues, with erythema, skin desquamation and fibrosis. Like resistance to radiotherapy, radiosensitivity is caused by the mutated *ATM* gene due to dysfunction of the protein implicated in the chain of reactions to which the AT protein belongs, some of which, like IGF-IR, EGF and Chk2, are responsible for radioresistance (Mamon et al., 2003; Takai et al., 2002; Yu et al., 2003; Sartor, 2003).

Tumour occurrence varies even among A-T patients in the same family, some of whom will develop multiple primary tumours, whereas others do not develop any. The link between A-T and the increased incidence of lymphoid and non-lymphoid tumours is due to the role of the ATM protein in protecting against DNA double-strand breaks (DSBs). The ATM defect abolishes the cell cycle checkpoints following a DSB, with inappropriate progression of the cycle and accumulation of damage until cell death (Taylor 2007). ATM-correlated tumorigenesis results from both an increased frequency of rearrangements of the chromosomes involving the loci of immunoglobulins or T-cell receptors on the one hand and the oncogenes on the other, and genetic events linked to the role *ATM* plays together with other tumour susceptibility alleles (*BRCA*, *p53*, etc.) (Ahmed 2006).

1.4 Biological markers

Among the analytes currently examined in the laboratory, serum α -fetoprotein (AFP) is the most reliable, being elevated in around 95% of A-T patients. Clinically A-T patients with normal AFP levels at molecular analysis either have an *ATM* gene mutation (rare, defined as ataxia without telangiectasia [A(-T)]) or an *APTX* gene mutation (common, AOA1). We think serum AFP determination is compulsory for the differential diagnosis of A-T.

The karyotypic mutation consists in an increased rate of spontaneous chromatid ruptures and the presence of rearrangements involving chromosomes 7 and 14 in the loci for the heavy chains of immunoglobulins and T-cell receptors.

Before molecular tests became available, radiosensitivity testing was used for the purposes of diagnosis. These have now lost their importance following observations that some A-T patients have reduced or almost normal sensitivity to ionising radiation, while non A-T individuals have radiosensitivity levels similar to those of A-T patients. In addition, radiosensitivity testing is arduous and requires a great deal of experience. Research laboratories performing radiosensitivity testing follow specific protocols developed over the years for both normal and pathological reference values such as the colony surviving assay (CSA), radioresistant DNA synthesis (RDS) and chromosome fragility tests performed using ionising radiation or radiomimetic compounds like bleomycin and neocarzinostatin.

Other laboratory tests, largely used for research purposes include cytofluorimetry to measure the G1/G2 ratio in the cell cycle, and the neutral and alkaline comet assay to determine the severity of DNA damage.

The most reliable molecular marker for A-T diagnosis is universally considered to be the determination of ATM protein in stabilized lymphoblastoid cell lines from patients with a clinical diagnosis of A-T. Protein levels are reduced (less than 20%) or absent in 99% of

patients with *ATM* gene mutations. Protein determination takes four to six weeks for stabilization of the lymphoblastoid line and another week for Western blotting analysis. Mutation analysis is done by different methods to identify the possible different types of mutation: DHPLC on encoded exons, followed by direct sequencing of the region with the abnormal chromatographic pattern; transcript sequencing; multiple ligation of probe amplification (MLPA).

1.5 Differential diagnosis

The autosomal recessive ataxias form heterogeneous groups of diseases sharing other characteristic neurological and clinical disorders in addition to cerebellar ataxia (Di Donato et al., 2001; Koenig, 2003) ([see table](#)).

A-T itself may present with different clinical signs, sometimes making it difficult to establish certain diagnosis on the basis of clinical features alone. In these situations biochemical-molecular tests are very helpful for the purposes of establishing a final diagnosis of A-T forms hitherto classified as “A-T variants” according to clinical and laboratory findings (Ying and Decouteau, 1981; Fiorilli et al., 1985; Taylor et al., 1987; Chessa et al., 1992). *ATM* gene mutations have also been identified in some of these patients (McConville et al., 1996; Gilad et al., 1998) thereby confirming the diagnosis of A-T. Mutations of the *Mre11* gene, a DSB repair gene belonging to the MNR complex, have been identified in patients with the clinical and laboratory features of A-T but lacking telangiectasias (A-T-like disorder) (Stewart et al., 1999; Pitts et al., 2001; Delia et al., 2004; Petrini, 1999). In addition, mutations of the *APTX* gene have been found in patients with neurological features very similar to A-T but with normal α -fetoprotein and lacking multisystem involvement, chromosome instability and increased sensitivity to ionising radiation, and defined as ataxia oculomotor apraxia (AOA).

All patients with these “A-T variants” have been investigated for the A-T phenotype at cell level (radiosensitivity) and by molecular (DHPLC and sequencing) and biochemical tests (Western blot). The discovery of mutations in the *Mre11* gene in patients with ataxia telangiectasia-like disorder (ATLD) and mutations in the *APTX* gene in patients with con AOA1, presenting the same neurological phenotype and age at onset as A-T patients, led to the production of a panel of antibodies against proteins Mre11, NBS1, Rad50 and APTX. This panel allows a first level differential diagnosis between pathological forms similar to A-T such as ataxia telangiectasia-like disorder, Nijmegen breakage syndrome and ataxia oculomotor apraxia types 1 and 2.

As a rule of thumb, if α -fetoprotein levels are normal in a patient with A-T-like neurological features, serum albumin and cholesterol should be measured for a differential diagnosis with AOA1. Thereafter it would be useful to contact the central laboratories responsible for molecular diagnosis (see below).

2. DIAGNOSTIC PROTOCOL

2.1 Inclusion criteria

Male and female persons of any age presenting the following clinical and laboratory features will be eligible for enrolment:

- ataxia /choreoathetosis
and/or
- oculomotor apraxia
- with or without telangiectasias

and

- elevated levels of serum α -fetoprotein

A registration form (**Form 1.01**) and a diagnosis form (**Form 30.01**) will be filled in for patients meeting these inclusion criteria. Annual follow-up forms (**Form 30.02**) will then be filled in and sent to the AIEOP/FONOP Centre in Bologna.

All subjects meeting the inclusion criteria will be enrolled in the Protocol.

2.2 Certain diagnosis

Certain diagnosis of A-T can only be established by:

- analysis of ATM protein expression (with evidence of reduced or absent protein expression)

or

- analysis of *ATM* gene mutation (with identification of the mutation)

2.3 Sending samples

On request of a centre in the network, the Rome Centre or Turin Centre will undertake analysis of levels of the ATM protein and correlated proteins and analysis of the *ATM* gene mutation for certain diagnosis of A-T. Before sending blood samples specific informed consent must be obtained from each family (see [attachments](#)) and kept by the centre in the network for each of its patient enrolled in the Protocol.

After making arrangements by telephone, the following must be sent for these tests:

- a 10-20 cc blood sample in heparin sodium

The samples must be sent at room temperature to one of the following laboratories:

ROME: Prof. Luciana Chessa

U.O.D. Genetica Medica
A.O. S. Andrea
Via di Grottarossa 1039
00189 Roma, Italy
Tel/fax: (39).06.33775258
luciana.chessa@uniroma1.it

TURIN : Dr. Alfredo Brusco
Dipartimento di Genetica
Università di Torino
Via Santena 19
10126 Torino, Italy
Tel. (39) 011.6334480
FAX (39) 011.6705668
e-mail: alfredo.brusco@unito.it

- Samples must be accompanied by **n° 1 National Health Service request form** duly filled in (date of sampling, patient details with place and date of birth, place of residence, health card number, tax code number, reason: molecular analysis for rare diseases: **RD040**).
- Samples must also be accompanied by **Form AT/A** duly filled in and sent to the charge of the Coordinating Centre via TRACO 10 service which guarantees delivery of samples by 10 a.m. on the following day.
- Samples must be sent from **Monday to Wednesday** each week.
- The results of the test for protein levels will be notified within two to four months.
- The outcome of mutation analysis will be notified within six to eight months.

2.3 Tests to be performed at diagnosis and during follow-up:

At diagnosis:

- α -fetoprotein
- Haemochrome
- Lymphocyte subpopulations (CD3, CD4, CD8, CD19)
- T-cell proliferation to mitogens (PHA) (optional)
- IgG, IgA, IgM, IgE
- IgG subclasses (optional)
- Autoantibodies (ANA, ASMA, AMA, APCA) (optional)
- Glycaemia
- Cholesterol
- Albuminaemia
- SGOT, SGPT, gGT
- Karyotype on 50 metaphases to search for clonal aberrations
- Brain MRI
- Neurological assessment
- Disability scale: Vineland Index
Barthel Index (from 16 years)

- Spirometry (if the test can be performed)

During follow-up:

Every 6 months:

- Neurological and neurorehabilitation assessment

Every 12 months:

- α -fetoprotein
- Haemochrome
- Lymphocyte subpopulations (CD)
- T-cell proliferation to mitogens (PHA) (optional)
- IgG, IgA, IgM, IgE
- IgG subclasses (optional)
- Disability scale: Vineland Index
Barthel Index (from 16 years)
- Spirometry (if the test can be performed)
- Sputum culture

Tests to be performed on clinical indication:

- Abdominal ultrasound scan and/or MRI (for suspected lymphoma or other tumours)
- Brain MRI
- EMG and ENG*
- Echocardiography
- Holter ECG
- Blood gas analysis

*EMG and ENG (from the age of six years; before in case of emergence of clinical signs suggestive of peripheral involvement)

3. THERAPEUTIC RECOMMENDATIONS

The complexity of the different diseases sharing the same genetic mutation, and the heterogeneous clinical manifestations currently make it difficult to provide uniform therapeutic recommendations for all patients.

The most appropriate treatment strategies must be implemented depending on the prevalence of a particular problem or a series of different problems.

The following recommendations are listed for the management of different clinical problems.

3.1 Recommendations for the management of immunological problems and infections

Prophylaxis against *P. carinii*. Prophylaxis with co-trimoxazole must be considered in patients with a significant CD4+ deficiency. In these cases reference can be made to the recommendations suggested for immunodepressed children: prophylaxis should be started if CD4+ cells are $< 750/\text{mm}^3$ in children aged < 12 months; $< 500/\text{mm}^3$ in children aged between one and five years; $< 200/\text{mm}^3$ in children aged > 5 years. It should be emphasized that PCP infection is extremely rare and no controlled studies are currently available on the topic.

Vaccinations. For AT patients with a mild immune impairment there are no contraindications to administration of vaccines belonging to the vaccine calendar in use in Italy. Live attenuated vaccines can be administered if the proliferative response to mitogens is normal and the absolute number of CD4+ lymphocyte is $> 500/\text{mm}^3$. If these parameters are not met vaccination should be postponed.

Administration of purified polysaccharide-protein conjugate vaccines (tetanus, diphtheria, pertussis, hepatitis anti-H. influenzae, anti-influenza, anti-pneumococcus) is recommended in all subjects as they are not harmful and trigger an antibody response depending on the intensity of the immune deficiency.

In particular, systematic use of active immune prophylaxis with conjugate vaccines is recommended: anti-pneumococcus; anti-meningococcus; anti-H. Influenzae. The vaccine schedule should be based on the patient's age and the vaccine characteristics.

- Conjugate anti-pneumococcal vaccination is recommended: for children under one year of age, two administrations one month apart and a third dose in the second year of life; for children over one year, two administrations with at least a two month interval in between the doses.

- Conjugate anti-meningococcal vaccination is recommended: for children under one year of age, three administrations at one month intervals; for children over one year, a single administration.

- Conjugate anti-H. influenzae vaccination is recommended: for children under one year of age, three administrations (according to the vaccine calendar in use in Italy); for children over one year, a single administration.

Antibiotic prophylaxis. AT patients may have an increased susceptibility to infections due in part to the immune defect and in part to "mechanical" problems (pneumonia ab ingestis cause by impaired swallowing; diminished chest expansion and difficulty walking due to neurological problems).

In any case prompt aggressive antibiotic therapy is recommended to control acute episodes of infection. In case of recurrence antibiotic prophylaxis can be implemented for a duration to be established for each patient by the treating physician.

Immunoglobulin replacement therapy

The humoral immune defect is usually mild and does not require immunoglobulin replacement therapy unless the patient is clinically symptomatic, namely with a high frequency of airway infections.

Of course this treatment is indicated in forms of A-T:

- with severe hypogammablobulinaemia;
- with a severe T-cell deficiency similar to that seen in SCID.

Therapy should be administered and monitored according to the guidelines provided in the Recommendations for XLA and CVID.

3.2 Recommendations for the management of neurological and neurorehabilitation problems

A-T is a slowly progressive neurodegenerative disease leading to loss of major adaptive functions, namely neuromotor functions, in the space of a few years due to loss of many neurological mechanisms involved in postural control, fine coordination tasks and visual exploration of the environment. As in other CNS disorders, the reduced motor autonomy and ultimately the inability to walk unaided mark an accelerated disease progression because they reflect not only disease evolution, but also the ensuing cascade of effects in terms of residual neurological skills and the patient's general condition.

Like all degenerative conditions, lacking an aetiological treatment the aim of symptomatic therapy (rehabilitation and drug management) is to postpone the loss of basic neuromotor skills for as long as possible or create the conditions for patients to recover neuromotor function or compensate so as to enhance adaptation and autonomy. Each neurological function is sustained by a scaffolding of mutually harmonized skills and automatisms. A functional loss appears when these elements deteriorate beyond the point of internal substitution by the system. Dwindling function can sometimes be postponed by means of aids acting as simplified replacements of components of functional organization. At other times these same aids may allow relearning via alternative original routes. Dedicated rehabilitation measures should be evaluated early on for A-T children to combat critical periods in disease evolution.

Certain critical stages mark disease evolution and require specific rehabilitative interventions:

- a) The emergence of truncal ataxia as a factor limiting neuromotor development (second year of life);
- b) Supervening oculomotor apraxia (third to fourth year of life) having a cumulative effect on the preceding difficulty undermining postural stability, stability of reference points in the visual environment, quality of visual exploration and implicit representation of the environment;
- c) Progressive motor slowing (bradykinesia and hypokinesia) making the patient's adjustments to adapt to changes in environmental conditions increasingly late and

ineffective (fifth to sixth year of life) and lastly the loss of the ability to stand and walk unaided (< ten years);

- d) Difficulties in communication and social interaction linked to loss of coherent speech and writing skills (sixth to ninth year of life).

Multidisciplinary intervention must be planned for each of these stages and symptoms bearing in mind the specific needs of the individual patient. Intervention should aim at: a) the use of motor functions affected by the disease in a meaningful setting enhancing the single anti-gravitational and coordination skills constituting their essential elements; b) an individual search for compensation strategies and mechanisms; c) the development and adaptation of aids to replace individual skills or functional modules no longer subject to recovery.

Lastly, many A-T patients present neurological symptoms that may benefit from specific drugs (postural tremor, parkinsonian signs, hypokinesia/brachykinesia, choreic or choreo-athetotic dyskinesias, etc.) that should be prescribed adjusting the dose in relation to the individual patient and the symptom to be targeted by the treatment.

3.3 Recommendations for the management of respiratory problems

Considering mainly the likelihood of “mechanical” problems (pneumonia ab ingestis due to impaired swallowing; diminished chest expansion and difficulty walking due to neurological problems), it is important to assess the need to devise a programme of respiratory physiotherapy tailored to the individual patient.

3.4 Recommendations for the management of other problems (diet/gastroenterological problems, ENT, etc.)

The appropriate specialists should be involved in the management of specific problems such as gastroenterological, otolaryngological conditions, etc.

3.5 Recommendations for radioprotection

The radiosensitivity of A-T patients emerged dramatically for the first time in 1968 with a fatal reaction to antitumoral radiotherapy (Morgan 1968). For this reason, treatment with ionising radiation and radiomimetic drugs is absolutely contraindicated in A-T patients. A-T patients presenting tumours must be treated using attenuated chemotherapy protocols.

Although accurate epidemiological information is not currently available on the risk of developing tumours related to exposure of A-T patients to radiodiagnostic procedures, diagnostic ionising radiation (standard x-ray, CT scans, scintigraphy) must only be used when strictly necessary. Whenever possible, radiodiagnostic techniques should be replaced by ultrasound or magnetic resonance imaging.

Heterozygous carriers of *ATM* mutations have an in vitro radiosensitivity in between that of normal subjects and homozygous A-T individuals (Neubauer 2002). However, heterozygous A-T carriers do not appear to have an increased sensitivity to radiotherapy (Bremer, 2003) and hence can be treated using standard protocols.

3.6 Attenuated chemotherapy

Leukaemia and lymphomas can be treated and effective chemotherapy protocols are available for use in A-T patients. However, as noted above the radiosensitivity of A-T patients is an absolute contraindication to treatment with ionising radiation and radiomimetic drugs. In case of tumours attenuated chemotherapy protocols must be instituted in agreement with the AIEOP and adult Haematology Centres.

3.7 Drugs trials currently underway

The well-established evidence that A-T cells are exposed to continuous oxidative stress suggested the possibility of treating the *ATM* mutation with exogenous antioxidants: experimental trials are currently underway.

4. PREVENTION

4.1 Prenatal diagnosis and identifying disease carrier status

Identifying *ATM* gene mutations is important not only to understand the molecular mechanisms underlying the disease, but also for prenatal diagnosis and genetic counselling. Prenatal diagnosis was performed long before the advent of molecular analysis by radiosensitivity testing of foetal cells. This approach often led to false negative results (personal observation). More recently, linkage analysis has been successfully used in patients whose mutations had not been identified. Direct molecular prenatal diagnosis offers 100% accuracy rate but is only possible when the familial mutations are known (Chessa et al., 1999).

Molecular segregation. Identifying carriers among A-T family members has always been a focal point of research both for the possible increased predisposition to cancer and cardiovascular diseases and for the need to offer in-depth genetic counselling. The increased sensitivity to ionising radiation also affects heterozygous carriers, albeit to a lesser extent than A-T patients, but the accuracy of identifying health carriers by this method does not exceed 85% [45], making it unsuitable for diagnostic purposes. Identification of the *ATM* gene and its mutations in an affected individual in an A-T family has fostered molecular segregation studies to disclose which siblings are heterozygous carriers in addition to the parents. In the rare cases in which the underlying mutations cannot be identified, haplotype analysis with microsatellite markers will yield a result with a confidence interval in excess of 98%.

Predisposition to cancer. The predisposition of healthy carriers of *ATM* mutations to develop tumours remains controversial and is the topic of debate among disease experts. Michael Swift's pioneering studies provided epidemiological evidence of a significant increase in breast cancer among the close relatives of A-T patients (Swift et al., 1987). Data collected in Italy indicated a greatly increased risk of stomach cancer in A-T families with respect to the general population (Chessa and Fiorilli, 1993). Even the molecular identification of A-T heterozygous carriers has failed to clarify the issue fully despite attempts to form a consortium of researchers worldwide to reach a significant number of A-T families. Renwick et al. (2006), studying 443 families with breast cancer and 521 controls, recently demonstrated that *ATM* gene mutations give heterozygous carriers a 2.37 relative risk of developing cancer. *ATM* is thus a minor factor of genetic susceptibility to breast cancer.

4.2 Sending samples

At the request of centres in the network, the Rome Centre or the Turin Centre will undertake analysis of the *ATM* gene mutation to identify carrier status in the parents and close relatives of A-T patients. If the proband's mutations cannot be identified, molecular analysis can be performed on family members by reconstruction of the disease haplotype with informative markers. Before sending blood samples specific informed consent must be obtained from each family and kept by the centre in the network for each of its patient enrolled in the Protocol.

After making arrangements by telephone, the following must be sent for these tests:

- a 20 cc blood sample in heparin sodium
- or
- a 7 cc blood sample in EDTA

The samples must be sent at room temperature to one of the following laboratories:

ROME: Prof. Luciana Chessa
U.O.D. Genetica Medica
A.O. S. Andrea
Via di Grottarossa 1039
00189 Roma, Italy
Tel/fax: (39).06.33775258
luciana.chessa@uniroma1.it

TURIN: Dr. Alfredo Brusco
Dipartimento di Genetica
Università di Torino
Via Santena 19
10126 Torino, Italy
Tel. (39) 011.6334480
FAX (39) 011.6705668
e-mail: alfredo.brusco@unito.it

- Samples must be accompanied by **n° 1 National Health Service request form** duly filled in (date of sampling, patient details with place and date of birth, place of residence, health card number, tax code number, reason: molecular analysis for rare diseases: **RD040**).
- Samples must also be accompanied by **Form AT/A** duly filled in and sent to the charge of the Coordinating Centre via TRACO 10 service which guarantees delivery of samples by 10 a.m. on the following day.
- Samples must be sent from **Monday to Wednesday** each week.
- The test results will be notified within two to four months.

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FORM A/ AT

Patient Surname _____ Name _____

Date of birth
 day month year

Referring doctor:
 Istitution of origin.....
 Via.....
 Post code..... City.....
 Tel..... Fax.....
 e-mail.....

Requests:

- analysis of ATM protein (with evidence of reduced or absent protein expression)

- analysis of the *ATM* gene mutation (with demonstration of the mutation)

Send to: _____

Place of dispatch _____ Date of dispatch _____

Signature _____

Form B / AT

Informed consent to intravenous/subcutaneous immunoglobulin treatment of minors

I/We the undersigned.....parent/s
of.....
born in() on..... have been informed by
Dr./Prof..... that the clinical conditions of my/our
daughter/son require treatment with immunoglobulins and that such treatment is
not completely free of risks (including the transmission of hepatitis, etc.) I/we
have fully understood what has been explained to me/us by
Dr./Prof..... both in terms of clinical conditions and the risks
linked to the treatment and those which could arise by not receiving the treatment.
I/we therefore **consent / do not consent** (*) to my/our daughter/son receiving at
this institution the immunoglobulin treatment required throughout his/her
disease course.
(*) delete as appropriate.

Date..... Signature.....

Withdrawal of informed consent

I/We the undersigned parent/s of born in
..... () on**withdraw** my/our consent to immunoglobulin
treatment signed by me/us on
date..... Signature.....

Informed consent to intravenous immunoglobulin treatment of adults

I, the undersigned.....born in.....()
on..... Have been informed by Dr./Prof..... that
my clinical condition requires treatment with immunoglobulins and that such
treatment is not completely free of risks (including the transmission of hepatitis,
etc.) I have fully understood what has been explained to me by
Dr./Prof..... both in terms of clinical conditions and the risks
linked to the treatment and those which could arise by not receiving the treatment.
I therefore **consent/do not consent** to receive at this institution the
immunoglobulin treatment required throughout my disease course.
(*)delete as appropriate.

Date..... Signature.....

Withdrawal of informed consent

I the undersigned born in () on
.....**withdraw** my consent to immunoglobulin treatment signed by me on
.....

Date..... Signature.....

Form C / AT

Patient's Surname: _____ Name _____

Date of birth I_I_I_I_I_I_I
day month year

Referring physician :

Institution.....

Address

post code..... City.....

Tel..... Fax.....

e-mail.....

Surname _____ Name _____

Family relationship with the patient _____

Requests:

- analysis of ATM protein (with evidence of reduced or absent protein expression)
- analysis of the *ATM* gene mutation (with demonstration of the mutation)

Send to: _____

Place of dispatch _____ Date of dispatch _____

Signature _____