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RESTLESS LEGS SYNDROME (RLS) SECONDARY TO END STAGE RENAL DISEASE: CLINICAL FEATURES, PATHOPHYSIOLOGY, AND GENETICS.

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Sinceramente e semplicemente grazie,

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Summary

Chapter 1: Introduction

The term restless legs syndrome (RLS) was firstly introduced by Karl-Axel Ekbom in his descriptions of peculiar and tormenting paraesthesias occurring deep in the legs of a number of his outpatients (Ekbom, 1944; Ekbom, 1945; Ekbom, 1960). Afterwards, however, Macdonald Critchley disclosed that the English physician Sir Thomas Willis had already described symptoms resembling RLS in the 17th century (Critchley, 1955; Willis, 1685). In his case series, Karl-Axel Ekbom recognized that many sufferers had close relatives with creeping sensations in the legs, thus pinpointing that hereditary factors could play an aetiological role, and that RLS was probably a common condition with an approximate prevalence of 5%. Moreover, he also described RLS in the course of pregnancy, as well as a symptom of iron deficiency anaemia (Ekbom, 1960), and in blood donors (Ekbom, 1956), suggesting a potential role for iron metabolism.

Diagnosis of RLS: clinical criteria

The diagnosis of RLS is based on the clinical features of patients' symptoms. Indeed, RLS is a condition consisting of an urge to move mainly focused in the legs, usually accompanied by other abnormal sensations (e.g. burning, creeping, "internal itch", "jittery", "like insects crawling inside the legs"), that are relieved by movement as well as worsened by periods of rest and inactivity, and have a strong circadian occurrence being present or worse in the evening and night (AASM, 2005). However, some people describe only an urge to move, being unaware of the sensory component, while others localize the disorder also in the arms (up to 50% of cases) and/or other body parts (Michaud, 2000). Similarly, most patients describe a complete and immediate relief with movements such as stretching, flexing, or walking, and the severe cases not responding to movement usually recall this feature earlier in the disease course. One of the core features of RLS is the circadian occurrence at evening/night that could be influenced by different contributing factors: 1, the increase of

sleepiness, mirrored by the clinical complaint of worse symptoms in tired or sleep deprived conditions; 2, symptoms may worsen in association with a decrease in central nervous system activity or alertness, and conversely the engagement in conversations actually reduces symptoms severity; 3, in the evening the motor activity is reduced compared to daytime; and 4, the evening worsening may reflect an intrinsic circadian rhythm of RLS symptoms. Studies performed in constant routine protocols confirmed the circadian rhythm of RLS sensory and motor symptoms, with the intensity peak occurring after midnight, on the falling limb of the core body temperature rhythm and in association with changes in melatonin secretion and subjective alertness (Trenkwalder, 1999; Hening, 1999; Michaud, 2004).

In the last two decades the International RLS Study Group (IRLSSG) established (Walters, 1995), and then further reviewed the clinical diagnostic criteria for RLS diagnosis (Allen, 2003). The panel listed four essential criteria that should be all met for a positive diagnosis: 1) an urge to move the legs, usually accompanied by uncomfortable or unpleasant sensations in the legs; 2) the unpleasant sensations or the urge to move begin or worsen during periods of rest or inactivity such as lying or sitting; 3) the unpleasant sensations or the urge to move are partly or totally relieved by movement such as walking, bending, stretching, etc, at least for as long as the activity continues; and 4) the unpleasant sensations or the urge to move are worse in the evening or at night than during the day, or only occur in the evening or night. Additionally, the three following supportive criteria were also proposed: 1) positive response to dopaminergic treatment; 2) periodic limb movements (during wakefulness or sleep); and 3) positive family history of RLS suggesting an autosomal dominant mode of inheritance. The panel of expert also depicted three typically associated features encompassing the natural clinical course, the RLS-induced sleep disturbance, and the expected findings at medical evaluation or physical examination. The clinical course may vary considerably and seems related to the age-at-onset: when RLS starts before the age of 45-50 years old, the course is often insidious with intermittent symptoms that progress slowly up to daily occurrence of

paresthesias around 55-60 years old; conversely, late-onset RLS (i.e. after 50 years old) has a more abrupt and severe presentation that rapidly progresses over five years to a stable pattern. Moreover, in some patients RLS can be an intermittent disturbance and may spontaneously remit for years. RLS generally disturb nocturnal sleep and patients frequently seek for medical attention for this reason. The physical examination is generally normal, but may add information for those conditions that may be comorbid or secondary causes of RLS. Indeed, special consideration should be devoted to the assessment of iron status, as long as decreased iron stores are significant risk factors, and to the search for peripheral neuropathy or radiculopathy, possible associated features deserving different treatment strategies (Allen, 2003). Moreover, according to the International Classification of Sleep Disorders - second edition, the condition (i.e. idiopathic form) should not be better explained by another current sleep disorder, medical or neurological disorder, mental disorder, medication use, or substance abuse disorder (AASM, 2005).

Impact of RLS on nocturnal sleep and daytime vigilance

As outlined above, RLS is a circadian disorder that mainly affects patients at evening or at night, culminating between midnight and 2 a.m. (Trenkwalder, 1999; Hening, 1999; Michaud, 2004).

Along with disease course, and especially in treated patients suffering from augmentation, the circadian rhythmicity can be overridden with symptoms being present 24 hours a day (García-Borreguero, 2010).

Besides the primary symptoms, most RLS patients complain of poor nocturnal sleep. Most patients report difficulties in falling asleep for the occurrence of RLS symptoms, whereas other subjects fall asleep rapidly but awaken soon thereafter with unpleasant leg sensations that force them to get up and walk around to relieve their discomfort (Montplaisir, 1997). Considering either the presence of sleep onset (85%) or maintenance (86%) complaints, 94%

of patients had subjective poor sleep quality. Sleep laboratory studies confirmed that RLS patients had severe sleep disruption compared with controls, with objective evidence of longer sleep latency, reduced sleep efficiency and total sleep time) (Montplaisir, 1997; Hornyak, 2007).

A peculiarity of nocturnal sleep in patients with RLS is the frequent occurrence of repetitive limb movements during sleep (PLMS). Initially described as a specific sleep disorder by Symonds in a group of subjects presenting with nocturnal myoclonus of different origin (Symonds, 1953), PLMS were subsequently described in patients suffering from RLS and called "nocturnal myoclonus" in honour of Symonds, disregarding the fact that the features of these rhythmic contractions did not fulfil the time-length criteria for a myoclonus (Lugaresi, 1965). PLMS are now a well-recognized intrinsic feature of RLS, being present in 80% to 90% of the patients (Hornyak, 2007; AASM, 2005). PLMS are repetitive, highly stereotyped movements involving mainly the legs during sleep. The electromyographic features of PLMS, including their duration and periodicity, have been extensively studied, leading to characteristics that make their identification reproducible in the field of sleep medicine (Zucconi, 2006; AASM, 2005). To date, PLMS can be regarded as the only objective measure of RLS, given their correlation to other subjective severity measures demonstrated at least in some studies of RLS sufferers (García-Borreguero, 2004). Despite the fact that most of RLS patients actually had PLMS, we must consider that PLMS can be found in several other sleep disorders such as narcolepsy, REM sleep behaviour disorder, and periodic limb movement disorder (AASM, 2005), being therefore sensitive, but not specific, markers of RLS. However, the intrinsic periodicity features and overnight distribution of PLMS are significantly different in the different sleep disorders (Ferri, 2006; 2006b; Manconi, 2007).

Approximately one third of RLS patients also complain of daytime sleepiness or fatigue, probably related to the disruption of nocturnal sleep given the amelioration of daytime symptoms under dopaminergic treatment (Montplaisir, 1996; Kallweit, 2009). On the other

hand, it is even more surprising that most of the RLS patients do not complain of excessive daytime sleepiness despite their disruption of nocturnal sleep.

The burden of RLS: psychological and medical consequences

RLS may cause significant morbidity by several routes, ranging from the discomfort of the symptoms occurring in the evening to the potential sleep disruption and deprivation in the most severe cases. The REST studies evaluated in the setting of primary care of European and American adult population the incidence and impact of RLS. The RLS sufferers reported to suffer both directly for their bothering symptoms (e.g. pain, discomfort) and for sleep related problems. Among the most frequent consequences of their symptoms, we must mention lack of "energy" – fatigue (60%), difficulty sitting still or relaxing (57%), as well as depressed mood (54%), and impaired concentration (49%) (Hening, 2004; Allen, 2005).

RLS may also finally result in an impairment of quality of life. According to the SF-36, a standard measure used to assess health-related quality of life in 8 domains, both physical and mental areas can be explored. Patients with RLS obtained significantly lower scores (i.e. worse quality of life) for all the 8 domain of this assessment tool (Allen, 2005; Kushida, 2007), particularly for the physical domains which are on average one-standard deviation below from mean population values (Kushida, 2007).

Several studies also suggested that the burden of RLS may extend to significant psychiatric and somatic disorders, thus becoming a risk factor for further significant illnesses. At least three studies performed in Germany and in the United States disclosed that the lifetime and one-year prevalence of depressive and anxiety disorders are significantly more elevated in the RLS versus non-RLS sufferers, including groups of subjects suffering from other chronic medical conditions such as arterial hypertension (Rothdach, 2000; Sevim, 2004; Winkelman, 2006; Lee, 2008). These studies shared a cross-sectional design, and did not allow to establish a causality link between RLS and the combination of psychiatric dysfunction. However, RLS

patients were more likely to attribute their psychiatric disorders to their symptoms compared to the other illness controls (Winkelman 2005), and the most frequent depressive symptoms seemed related to the sleep disturbance experienced by most RLS sufferers (Hornyak, 2005).

Epidemiological studies have also suggested a link between RLS and physical illnesses (Phillips, 2000; Ulfberg, 2001; Winkelman, 2006; 2008). In particular these cross sectional studies found associations of RLS with diabetes mellitus (Phillips, 2000), heart problems and arterial hypertension (Ulfberg, 2001), and cardiovascular diseases (Winkelman, 2006). Notably, the latter association disclosed in the Wisconsin sleep cohort was found to be more robust in patients with more severe RLS in terms of symptoms occurrence (Winkelman, 2006), and was further replicated in the Sleep Hearth Health Study (Winkelman, 2008). In this context RLS appeared associated with both coronary artery disease and cardiovascular diseases after controlling for a complete range of cardiac risk factors (Winkelman, 2008).

Pathophysiology of RLS: evidences and hypotheses

RLS pathophysiology is certainly complex and multifactorial, with evidences suggesting that RLS may be regarded as a "network" disorder. Considering RLS features, encompassing the abnormal perception of sensory symptoms, the relief induced by movements, and the circadian occurrence, we may speculate that different regions of the nervous system could be involved from the periphery to the cortex. This anatomical complexity is reflected by the many different topographical, genetic, and biochemical causes of RLS that have been documented in isolation or in combination in different studies, as well as by the different treatment strategies that proved efficacy against the disorder with particular attention to the response to dopaminergic agents. A complete review of the different hypotheses underlying RLS pathophysiology is not the main aim of the current dissertation, and here below we will briefly mention the main lines of evidences on this topic.

The dopamine is certainly involved in RLS pathophysiology, as demonstrated by the impressive efficacy on RLS symptoms and motor features (Allen, 2003; Scholz, 2011). Neuronal interactions are modulated by dopaminergic transmission at very low doses at the level of the spinal cord and of the brain (excitability) (Heckman, 2009; Rizzo, 2009), and in patients with RLS this reduction of cortical plasticity can be restored by dopaminergic treatment (Rizzo, 2009). Moreover, in patients with RLS the soleus H-reflex recovery curves showed increased facilitation and depressed late inhibition, signs that indicate a diminished inhibition probably due to altered function of the descending spinal tracts, peripheral influences or changes in the interneural circuitry at the spinal level itself, or to combinations of these mechanisms (Rijsman, 2005). Given the evidences of an involvement of the spinal cord (Provini, 2001) and of the possible influence of supraspinal modulation of spinal cord excitability that is clearly influenced by dopamine, some authors suggested a key role for the dorsal- posterior hypothalamic area called A11 (Clemens, 2006). According to this medical hypothesis, the dopaminergic A11 area with its complex interactions with the neocortex and with the spinal circuitry may modulate sensory input and motor output at the spinal level in relation to highest brain functions. Moreover, the circadian rhythm of dopamine metabolites in patients with RLS showed a significant reduction of these compounds when measured at the cerebrospinal fluid level in the hours of RLS symptoms occurrence (Poceta, 2009).

Impairment of sensory perception was also found in RLS patients with static hyperalgesia that may be mediated by central sensitivization of A-delta-fiber mechanoceptor input, such as in neuropatic pain (Stiasny-Kolster, 2004), and that is reversed by dopaminergic treatment. Several objective evidences also suggest a peripheral origin of RLS sensory component, as documented by the presence of subclinical axonal sensory neuropathy at nerve and skin biopsy studies in idiopathic RLS patients (Iannacone, 1995; Polydefkis, 2000).

However, peripheral somatosensory input is not a necessary precondition for the syndrome, given the evidence provided by RLS symptoms occurring in amputees of lower extremities

(Hanna, 2004), but responsive to dopaminergic treatment. Therefore, central pain perception could play an important role. Accordingly, central opioid receptor binding did not globally differ in patients versus controls, but regional negative correlations between ligand binding and RLS severity in areas serving the medial pain system (medial thalamus, amygdala, caudate nucleus, anterior cingulate gyrus, insular cortex and orbitofrontal cortex) were disclosed (von Spiczak S, 2005). Moreover, RLS well respond to opioid treatment, with reversal of this response with concurrent use of naloxone, that is an opiate receptor blocker (Montplaisir, 1991). Interestingly, the effect of L-dopa does not seem to be secondary to that of opioids, as long as blockade of opiate receptors by naloxone does not alter the therapeutic effect of L-dopa, whereas the blockade of dopamine receptors attenuates the effect of codeine on RLS symptoms.

Another strong line of evidences links RLS pathophysiology to iron metabolism, encompassing the role of iron deficiency as a risk factor for symptoms occurrence from a clinical standpoint (Ekbom, 1960; O'Keeffe, 1994), and the good response of RLS symptoms to iron supplementation (Earley, 2004). Different studies attempted to assess iron metabolism in RLS patients and found correlations between symptoms severity and low ferritin concentrations (Earley, 2004), especially when the latter were measured at the cerebrospinal level (Earley, 2000). The evidence of reduced cerebrospinal ferritin and increased transferrin in the absence of similar findings in the serum strongly suggests a central nervous system iron deficiency occurring despite normal peripheral iron levels (Earley, 2000). Moreover, magnetic resonance imaging studies for regional iron content disclosed reduced iron in the substantia nigra and putamen in RLS patients versus controls (Allen, 2001), whereas autoptic studies confirmed a complex pattern of reduced iron and ferritin with increased transferrin as expected in iron deficiency conditions, with paradoxically decreased tranferrin receptors in the substantia nigra suggesting a specific abnormality in the regulation of transferrin receptor (Connor, 2003). Finally, iron is important for dopaminergic transmission within the nervous

system because it is an important cofactor for tyrosine hydroxylase, the step-limiting enzyme for dopamine synthesis, and also plays a role in the functioning of postsynaptic D2 receptors.

Finally, strong evidences for a key role of genetic predisposition have been documented in the last decades. Linkage studies in families with recurrence of RLS disclosed to date at least eight loci that however could not causally explain RLS occurrence (Trenkwalder, 2009). The RLS loci from RLS1 to RLS8 are mapped on the following chromosomes: 12q, 14q, 9p, 20p, 2q, 4q, 17p, and 19p. In addition to these loci, there is further evidence of genetic heterogeneity. Recently, genome-wide association studies detected gene variants that contribute to the risk for RLS in four chromosomal regions containing five genes (Stefansson, 2007; Winkelmann 2007; Schormairm 2008). One study showed associations with lower iron stores (Stefansson, 2007), but none of the genes conferring risk for RLS was related to iron, dopamine, or endogenous opioid systems. Conversely, MEIS1, BTBD9, MAPK2K5, LBXCOR1, and PTPRD genes seem largely related to embryonic development, and functional relations with RLS have not yet established.

Primary and secondary RLS

RLS and PLMS have been described in the context of several medical conditions, but only in few of them a strict relation has been proved. We will briefly discuss the main clinical associations of RLS, with the aim to introduce the rationale for the current research project. RLS may be an isolated disturbance (i.e. idiopathic RLS), but may also occur in the course of other clinical conditions (e.g. pregnancy) or diseases (anemia, peripheral neuropathy, uremia, neurological disorders). When a temporal link between a medical condition/disease and RLS is documented, then RLS is considered "secondary" to the condition itself. The final evidence of the association would require, however, the demonstration of the disappearance of RLS symptoms after resolution of the primary medical disease. The secondary forms of RLS are of high interest, because they may shed further light on the RLS pathophysiology, providing

useful information on the role of specific conditions inducing the occurrence of symptoms. In this context we believe that RLS pathogenesis is certainly multifactorial, and different factors may contribute to overcome an hypothetical “threshold” leading to the occurrence of clinical symptoms.

Since early descriptions of RLS, the association with iron deficiency anemia was reported (Ekbom, 1960), and this datum has been repeatedly confirmed by other clinical conditions that share the tendency to develop iron deficiency such as gastric surgery (Banerji, 1979) and repeated blood donations (Kryger, 2003; Ulfberg, 2004). As discussed above, iron deficiency has been recognized as a definite risk factor for RLS, and abnormalities of iron metabolism are currently considered key pathophysiological determinants of RLS.

RLS may be associated with peripheral neuropathies such as amyloid (Salvi, 1990), crioglobulinemic (Gemignani, 1997), uremic (Callaghan, 1966), and in some cases RLS symptoms could be the initial clinical manifestation of the peripheral nerve disturbance (Salvi, 1990). When a population affected by peripheral neuropathy is investigated for the potential occurrence of the syndrome, RLS is frequent in acquired polyneuropathy of sensory type and mild entity, mainly in women (Gemignani, 2006). Moreover, the involvement of subtle peripheral nerve abnormalities has been considered within the factors involved in RLS pathophysiology.

RLS has been associated with diabetes mellitus , and evidences for a potential role of peripheral nerve abnormalities in this context also emerged (Merlino, 2007). Moreover, in the context of RLS and peripheral diabetic neuropathy, a close link with the small fiber neuropathy subtype has been described (Gemignani, 2007), and further mirrors the data disclosed in otherwise idiopathic RLS (Iannacone, 1005).

Parkinson disease, a neurodegenerative movement disorder characterized by dopaminergic insufficiency, has been associated with RLS in some studies (Banno, 2000; Peralta, 2009), but apparently not confirmed by other authors disclosing prevalence rates comparable to the older

general population (Verbaan, 2010). According to the features of Parkinson disease and of RLS, the appearance of RLS symptoms at evening may be related to the cessation of effect of dopaminergic therapy, being therefore this class of patients potential mimic of RLS. Other chronic neurological conditions such as multiple sclerosis have been associated with RLS (Deriu, 2009; Italian REMS Study Group, 2008). In this context the association of RLS to higher pyramidal and sensory disability points to a potential role for abnormalities at spinal cord level (Italian REMS Study Group, 2008; Aydar, 2011), a finding that is mirrored by the association of RLS with spinal cord injuries (Telles, 2011; Mahovald, 2010), and reflects again the potential role of this part of the central nervous system in RLS pathophysiology. Similarly, RLS was found to frequently occur in patients suffering from stroke involving lesions of the subcortical brain areas such as the pyramidal tract and the basal ganglia-brainstem axis, further corroborating the above considerations (Lee, 2009).

RLS has also been associated with rheumatologic diseases (Hening, 2008). RLS has been described to frequently occur in the course of rheumathoid arthritis (Reynolds, 1986; Salih, 1994; Taylor-Gjevre, 2009), osteoarthritis (Taylor-Gjevre, 2009), and fibromyalgia syndrome (Yunus, 1996). However, some authors did not support this association when evaluated potential occurrence of serum abnormalities of these conditions in a cohort of RLS patients (Ondo, 2000), whereas other studies disclosed an association between the severity of rheumathoid arthtritis and RLS, and highlighted the potential role of alterations within the central nervous system at the level of the spinal cord or peripheral nerve (Salih, 1994), similarly to what was suspected for the association between RLS and multiple sclerosis (Italian REMS Study Group, 2008).

In the pediatric field, other two associations have been reported, despite the difficulty to ascertain RLS criteria in children. First, RLS may be associated with “growing pains” (Brenning, 1960). In particular, growing pains are supposed to disappear along with development, but some subjects that suffer from RLS in adulthood recalled these symptoms

during childhood. However, the actual literature needs more data to establish this association (Walters, 2002). Second, RLS may be associated with attention deficit hyperactivity disorder (ADHD). Accordingly, a bidirectional link between RLS and ADHD has been shown with evidence of increased prevalence of symptoms of inattentiveness in adolescents with ADHD (Kotagal, 2004), as well as increased prevalence of RLS or RLS symptoms in patients with ADHD (Cortese, 2005). These associations should be interpreted with caution given the difficulties of a RLS diagnosis in childhood and the different attention to ADHD in different countries and across time (Walters, 2008), but may reflect the effect of a complex genetic background (Schimmelmann, 2009).

Other clinical conditions have been associated with RLS in limited series of patients suffering from chronic lung disease, leukemia, Isaac's syndrome, Huntington chorea, amyotrophic lateral sclerosis, hypothyroidism, hyperthyroidism. However, most of these associations should be interpreted with caution given the high prevalence of RLS in the general population and the potential influence of several substances frequently used and known to worsen or induce RLS. Within the latter, we must mention antidepressants, lithium carbonate, dopamine receptor blocking agents such as classical neuroleptics.

Finally, RLS has been associated with conditions such as pregnancy that physiologically occur across lifespan. Accordingly, it has been shown that RLS can arise in the course of pregnancy, especially in the third trimester, and generally disappears reaching after delivery (Manconi, 2004). RLS in pregnancy is associated with lower values of hemoglobin and mean corpuscular volume and appear to be a transient form. However, women with transient RLS symptoms in the course of pregnancy have increased risk to reexperience these symptoms in future pregnancies as well as to develop the idiopathic form later in life (Cesnik, 2010).

Diagnosis of RLS: clinical "mimics"

Several studies applied the four essential diagnostic criteria in questionnaire forms in order to assess RLS prevalence in different epidemiological grounds, and frequently disclosed different pictures. Recently, Hening and co-workers evaluated the diagnostic pitfalls of the above mentioned diagnostic criteria and showed that many individuals satisfying these criteria actually have other conditions that simply "mimic" RLS (Hening, 2009). In particular, when extending the clinical investigation to other potential conditions such as leg cramps, positional discomfort, local leg pathology by means of concurrent questions, a percentage up to 16% of clear-cut non-RLS subjects appear as false positive. However, the concurrent search for these conditions using more extended questionnaires such as the Hopkins telephone diagnostic interview, and the atypical presentation of these "mimics" (e.g. painful sensations, constant association with cramps, relief with simple change of position, duration of the symptoms) may be of great help for a correct differential diagnosis (Hening, 2008; Hening, 2009).

Rationale for the study of uremic RLS

RLS has been initially described in association with uremic neuropathy (Callaghan, 1966) or anemia (Roger, 1991), two frequent comorbidities in patients with severe chronic renal failure requiring hemodialysis treatment, a condition frequently named "end stage kidney disease" (ESKD).

Several studies proved the higher prevalence of RLS in ESKD patients with different prevalence rates that were significantly higher compared to those of the general population and of control groups (Parker, Sleep Med Rev 2003). Moreover, RLS has recently been found to occur more frequently in patients with chronic kidney failure not yet requiring hemodialysis treatment compared to controls (Merlino, 2010). However, considering the associations disclosed in different studies with clinical (e.g. comorbidity, age, sex), kidney-related (hemodialysis features, dose, and duration), and biochemical (higher levels of different

electrolytes) parameters, most findings were disclosed in some studies but not replicated in the subsequent works, thus offering insufficient insight in disease pathophysiology. These features will be analyzed in detail in the discussion section of the dissertation.

The most striking feature of RLS in the course of ESKD is the resolution of the symptoms after successful kidney transplantation (Winkelmann, 2002), a finding that demonstrates the relation between renal function and RLS. However, any relation between RLS occurrence and intrinsic features of renal function have been found to date, and the relation between kidney disease and RLS still lacks of a mechanistic demonstration.

Moreover, the condition of ESKD shares different features that are by themselves considered causes of secondary RLS. Indeed, ESKD patients frequently suffer from peripheral neuropathy (Krishnan, 2009), and from anemia given the insufficient to absent renal production of erythropoietin. In this setting, we should also remind that several other clinical conditions such as leg cramps, pruritus, fatigue frequently occur and may mimic RLS (Murtagh, 2007), and the clinical studies focused on RLS did not simultaneously evaluated these features.

Finally, RLS has been associated with cardiovascular morbidity in the general population (Winkelman, 2008), as well as with increased mortality in ESKD patients (Winkelmann, 1996). However, the mechanistic link between RLS occurrence, cardiovascular morbidity, and survival has not been yet demonstrated neither in the general population nor in ESKD patients.

On the basis of the above considerations, we consider uremic RLS as an ideal disease model that could shed light on different mechanisms of RLS pathophysiology. The aim of the current PhD project is the systematic evaluation of RLS using validated tools, together with the documentation of clinical and sleep-related comorbidities, of a comprehensive assessment of kidney-related factors and peripheral neuropathy, of the impact of RLS on nocturnal sleep and daytime symptoms including quality of life and mood, and of its impact on the occurrence

of new cardiovascular events and on survival at follow up in a large homogeneous population of ESKD patients.

The research hypothesis underlying this PhD project is that RLS aetiology could be certainly regarded as multifactorial, encompassing different environmental factors together with a genetic predisposition. In this context, we may speculate whether the condition of ESKD could trigger the expression of a pre-existent genetic background, and whether uremic RLS could be a mixture of conditions encompassing forms with “idiopathic” and “secondary” features.

Chapter 2: Methods

The research project of this PhD has been performed with a collaboration between the Department of Internal Medicine, Aging and Renal Disease (Professor Gaetano La Manna, Professor Sergio Stefoni) and the Departmenf of Neurological Sciences (Professor Pasquale Montagna, Professor Giuseppe Plazzi) of the University of Bologna. The tutor of the current PhD project was Professor Montagna, who prematurely died on December 9th 2010.

The overall aim of the project was the evaluation of RLS, of its potential clinical risk factors, and of its impact on quality of life and survival, in a large cohort of consecutive patients suffering from End Stage Kidney Disease (ESKD) undergoing stable hemodialysis (HD) treatment from at least 3 months. The patients were recruited in two distinct dialysis units (S.Orsola Hospital, Professors Stefoni and La Manna; Maggiore Hospital, Dr. Claudio Campieri) during the first year (2008) of the project. The project was approved by the local ethic committee (code: SLEEP 09-01), and all patients recruited for the study voluntarily accepted to participate.

Inclusion criteria were willingness to participate to the study, stable HD treatment lasting at least 3 months, absence of acute intercurrent illnesses, of chronic psychiatric or cognitive disturbances, and absence of malignances including myeloma and lymphoproliferative disorders, cachexia or severe infection. One-hundred-sixty-two patients with ESKD undergoing long-term HD in two Dialysis Units in Bologna were consecutively recruited into the study from March 2008 to December 2008.

Data collection: baseline assessment and cohort definition

We collected demographic and clinical data from patients' records, we evaluated sleep complaints by means of a direct structured clinical interview and of validated questionnaires,

and we performed a neurological examination to assess the likelihood of peripheral neuropathy.

Clinical and laboratory features

Clinical characteristics of the population (age, sex, body mass index [BMI]) were systematically collected, including the evaluation of the comorbidity by means of two indexes, the Charlson Comorbidity Index (C-CI) (Charlson, 1987), and its adapted form for ESKD patients (ESKD-CI) (Hemmelgarn 2003). Indeed, an higher comorbidity load appeared to have a role in the determination of sleep disorders in HD patients in previous studies, or at least to be significantly associated with insomnia (De Santo, 2005).

The C-CI attributes a specific score ranging from 1 to 6 to a number of morbid conditions. In particular, myocardial infarction, congestive heart failure, peripheral vascular disease (including aortic aneurysm ≥ 6 cm), cerebrovascular disease (stroke with mild or no residua, or transient ischemic attack), dementia, chronic pulmonary disease, connective tissue disease, peptic ulcer disease, and mild liver disease (with no portal hypertension, including chronic hepatitis), diabetes without end-organ damage (excuding diet-controlled alone) have a score of one. Hemiplegia, moderate or severe renal disease, diabetes with end-organ damage (including retinopathy, neuropathy, nephropathy, or brittle-diabetes), tumor without metastases (excluded if > 5 years from diagnosis), leukemia (acute and chronic), and lymphoma have a score of two. Moderate or severe liver disease has a score of 3, whereas metastatic solid tumor and AIDS (not just HIV positivity) have a score of six (Charlson, 1987). This scoring system has been found useful to predict survival and outcomes in patients with ESKD, (Fried, 2001), and also showed better discriminating features compared to other comorbidity scores such as the Khan and Davies ones (Van Manen, 2002). However, as long as the ESKD population obviously have “severe renal disease”, and frequently other co-shared common comorbidities, we also adopted a modified version of the C-CI adapted for

ESKD patients (ESKD-CI) that attributes a different score to 12 of the 18 comorbidities assessed (Hemmelgarn 2003).

Moreover, we calculated the global number of cardiovascular events (CVE) by means of counting the total number of myocardial infarctions, cerebral strokes or peripheral artery occlusions that occurred before the enrollment in the study for patients belonging to S.Orsola-Malpighi Dialysis Unit.

We recorded the following kidney-related factors recorded were: cause of kidney failure (Glomerular, Interstitial, Diabetic, Vascular, Polycystic, ESKD), previous kidney transplantation, dialysis vintage (months from the start of HD) and dose (number and hours of HD sessions per week), residual diuresis (urine output during 24 hours), dialysis technique (grouped as diffusive or mixed diffusive-convective), dialysis shift (morning, afternoon, or evening shift), and dialysis efficacy by means of Urea Reduction Rate (URR), and single pool Kt/V (spKt/V). In the clinical practice of HD care, our nephrologists used to attribute the HD shift on the basis of patients' severity, with more unstable patients undergoing HD in the morning, when more medical doctors were inward, and less severe patients in the evening. Therefore, we decided to not analyze the role of HD shift on RLS, despite previous studies have showed conflicting results with a beneficial or absent effect of the evening HD (Hsu, 2008; Bastos, 2007).

The following laboratory analyses performed within 4 weeks from clinical assessment were collected: blood cell count, hemoglobin, hematocrit, iron status (ferritin, transferrin, iron, TIBC), standard biochemical indexes, electrolytes, and intact parathyroid hormone (i-PTH), and inflammation markers (ESR, CRP).

Assessment of RLS, of other sleep disorders, and of peripheral neuropathy

A semi-structured clinical interview on sleep complaints was conducted by a Neurologist trained in Sleep Medicine (F.P.) assessing the following sleep disturbances: the presence of

RLS and of reported first-degree relative family history of RLS according to the International RLS Study Group criteria (Allen, 2003), the presence of Nocturnal Legs Cramps, of Insomnia, of Sleep-Related Eating Disorder according to the International Classification of Sleep Disorders (AASM, 2005; Provini, 2009), the presence of nocturnal smoking (Provini, 2010), and of pruritus according to the Yosipovitch Questionnaire (Yosipovitch, 2001).

For each condition, we applied specific questions encompassing the requested criteria for a positive diagnosis together with additional questions according to clinical practice of sleep medicine.

For patients complaining of pruritus, we administered the Yosipovitch Questionnaire. It includes questions on clinical history of the symptom, on current antipruritic medication use, on its effect on nocturnal sleep, daily activities, and habits, on the coping strategies adopted against pruritus, on its impact on quality of life measures, on verbal descriptors of itch sensation and affective dimensions, on its severity by means of visual analogue scales, and on the areas involved by means of self-marking a body diagram (Yosipovitch, 2001).

The family history was assessed asking patients about the presence of RLS complaints in their first-degree relatives, and subsequently confirmed by means of a self-administered questionnaire or telephone interview of relatives.

We investigated the temporal relation between sleep complaints and kidney disease, the frequency of sleep disorders occurrence, and the RLS severity at the moment of the study by means of the international RLS rating scale (IRLSS) (Walters, 2003).

Given the possible intermittent course of RLS, we defined RLS "life prevalence" by the positive answer to the 4 diagnostic criteria, whereas RLS "current prevalence" required the positive answer to the 4 diagnostic criteria and an IRLSS score ≥ 4 with occurrence of the symptoms at least 2-3 times per week as defined by the answer to item 7 of IRLSS. Patients with RLS (either currently or previously occurring) were also asked specific questions on RLS course and on its impact on the HD procedure. In particular, we focused on RLS

symptoms start (in terms of age, as well as before any evidence of kidney disease, during the period of chronic kidney failure, or after the start of HD treatment), on symptoms worsening after HD start, on RLS course (intermittent versus continuous), on its impact on sleep quality and sleep onset/maintenance insomnia (using a visuo-analogue scale, VAS). Concerning the relation between HD and RLS, we assessed whether RLS differed in the days with or without HD both in terms of sensations and timing, whether RLS was a bothering symptom during HD (including a VAS, and its frequency), and whether RLS has ever forced the patient to ask a precocious interruption of the HD procedure (including frequency).

Insomnia and nocturnal legs cramps were considered "frequent" if occurring at least many times per month. Moreover, we assessed whether insomnia and nocturnal legs cramps started or worsened after the start of HD.

Each patient underwent neurological examination in order to establish the estimated likelihood of distal symmetric polyneuropathy, assessed according to the consensus criteria for clinical and epidemiological research studies (England, 2005). In particular, the presence of abnormal sensations was clinically investigated in order to assess neuropathic symptoms, then specific items from the neurological examination were considered (i.e. decreased or absent ankle reflexes, decreased distal sensation, distal muscle weakness or atrophy). These features allow the ordinal stratification of patients with absent, low, and high risk of peripheral neuropathy in the absence of electromyographic or electroneurographic investigations.

We also used several self-administered questionnaires to systematically investigate the potential occurrence of sleep-disordered breathing or of subjective daytime sleepiness, and to assess circadian preferences. The Berlin Questionnaire (BQ) was used for the risk stratification of Obstructive Sleep Apnea Syndrome (OSAS) (Netzer, 1999), and the Italian version of the Epworth Sleepiness Scale (ESS) for the evaluation of subjective trait sleepiness (Johns, 1991; Vignatelli, 2003). The BQ includes a set of questions on nighttime symptoms

(e.g. loud snoring), on daytime sleepiness, and on the presence of daytime hypertension and of overweight in the last 3 months, that allows the stratification of patients as having absent, low, and high risk of OSAS in the absence of nocturnal cardiorespiratory monitoring (Netzer, 1999). The ESS asks to assess with a score ranging from 0 to 3 the frequency of dozing off in 8 different situations in the last two weeks, a score ≥ 10 suggests Excessive Daytime Sleepiness (EDS) (Johns, 1991; Vignatelli, 2003). Circadian preferences were also assessed by means of the morningness-eveningness questionnaire (MEQ) (Horne, 1976). According to the MEQ, subjects can be classified as having a morning, intermediate, or evening circadian preference.

Assessment of subjective perception of fatigue, health-related quality of life, and depression

In order to assess the impact of RLS on different dimensions of patients' life, we self administered other standardized questionnaires on fatigue, health perception, and mood. Fatigue was assessed by means of the Fatigue Severity Scale (FSS) (Krupp, 1989). The FSS asks to score from 1 to 7 nine the agreement with different statements about the presence and effect of fatigue. This questionnaire proved efficacy in differentiating fatigue from depression, being fatigue an highly subjective symptom related to the effort required to accomplish a sustained task and having both central and peripheral components (Krupp, 1989; Chauduri, 2004). Health Related Quality of Life (HRQoL) perception was assessed by means of the Italian version of the SF-36 (Apolone, 2000). Briefly, the SF-36 measures eight different dimensions of health: physical function (PF), role limitations related to physical problems (RF), bodily pain (BP), general health perception (GH), vitality (VT), social functioning (SF), role limitations due to emotional problems (RE), and mental health (MH). The raw scores are linearly transformed into 0–100 scales. Higher scores indicate better health (Apolone, 2000). Mood was assessed by means of the Beck Depression Inventory-II (BDI-2) (Beck, 1966). BDI-2 includes 21 questions about how the subject has been feeling in the last week. Each

question has a set of at least four possible answer choices, ranging in intensity. The BDI-2 is a 1996 revision of the BDI (Beck, 1996b), developed in response to the American Psychiatric Association's publication of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, which changed many of the diagnostic criteria for Major Depressive Disorder. The BDI-2 is not intended as a diagnostic tool for depression, but to provide a quantitative assessment of the intensity of depression (Beck, 1966; Beck 1996b).

Data collection: follow-up evaluation

After being enrolled in the study and being evaluated with to the above mentioned procedures (baseline), the cohort of patients belonging to S.Orsola-Malpighi Dialysis Unit was followed for ESKD treatment by our colleagues. The enrolment in the study did not influence any decision on ESKD management.

At 18 months of follow-up, we evaluated the survival of each subject (including the causes of death), and the occurrence of new CVE compared to baseline in order to test the potential effect of RLS on ESKD outcomes together with the other common risk factors.

Statistical analyses

All data collected were explored with explorative statistical analyses for the whole population and for different subgroups (e.g. patients with and without RLS), using mean ± standard deviation and frequency for continuous and categorical variables respectively.

Patients with and without RLS, as well as patients belonging to different groups, were contrasted using non parametric statistical approaches. In particular, we applied Mann-Whitney U or Chi Square tests for continuous or categorical variables as appropriate. When more than two groups of subjects were compared, we applied Kruskall-Wallis or Chi Square tests, followed by Mann-Whitney U or Chi Square tests for post-hoc analyses. Fisher Exact test was used instead of Chi Square test when the observed frequency of a variable in a group

was less than 5%. We preferred a non – parametric approach in order to perform uniform analyses for both normally and non-normally distributed data. Variables significantly associated to RLS at baseline assessment were further tested as predictors of RLS (dependent binomial variable) with a multivariate logistic regression model approach.

Relations between different continuous variables at baseline (e.g. IRLSS score and ESS, FSS, SF-36 scores) were tested using Pearson correlation coefficient analysis.

Survival was analyzed by means of Kaplan Meier curves and Cox regression models. Kaplan Meier curves were constructed for patients with or without RLS for all-cause mortality and compared by log-rank test. Each factor significantly associated with clinical outcome (i.e. death, or occurrence of a new CVE) in the univariate analysis was included in two Cox regression models with occurrence of new cardiovascular events and mortality as the dependent variable. Interactions between RLS and clinical or laboratory variables were also tested by the Cochran–Mantel–Haenszel test. Two models were fit for each outcome, one including RLS classified as absent or present and a second including RLS classified as absent, intermittent and continuous.

A p-value < 0.05 was considered statistically significant. All analyses were performed using the statistical packages SPSS (version 15.0, SPSS Inc., Chicago, IL) and SAS (Version 9.1, SAS Institute Inc, Cary, NC).

Chapter 3: Results

One-hundred-sixty-two patients with ESKD undergoing long-term HD in two Dialysis Units in Bologna (100 at S.Orsola Hospital, and 62 at Maggiore Hospital) were consecutively recruited into the study from March 2008 to December 2008.

According to inclusion criteria for the study, 11 patients were excluded because of refusal to participate or presence of acute illnesses. However, no statistically significant difference concerning demographic and dialytic parameters was found between patients included (162) and excluded (11) in the study.

Baseline evaluation of the whole population: the clinical predictors of current RLS

Patients: demographic and clinical features

Patients were mostly males (65%), had a mean (± standard deviation) age of 66.5±14.3 years, a mean BMI of 24.8±4.4, a mean comorbidity load of 4.8±2 at C-CCI and of 2.8±2.3 at ESKD-CI, and a prevalence of type 2 diabetes of 26%.

The renal disease leading to ESKD was Glomerular in 20%, Interstitial in 11%, Diabetic in 19%, Vascular/Hypertensive in 28%, Polycystic in 7%, unknown/ESKD in 11%, and other causes in 3%. Twelve percent of patients had a previous kidney transplantation with subsequent graft failure.

Patients were treated with diffusive (79%) and with mixed (diffusive-convective) HD techniques (21%). Mean dialysis vintage was 39.8±44.6 months, with 2.8±0.5 dialysis sessions per week each one lasting 4±0.2 hours, and a dialysis efficacy estimated by a mean URR of 66.7±10.0 and a mean spKt/V of 1.4±0.4. Mean residual diuresis was 321.8±463.2

mL/48h, with 25% of patients having more than 500mL/48h, 18% between 0 and 500 mL/48h, and 57% lacking any residual diuresis.

Concerning treatments, no patient has never assumed drugs known to treat or affect RLS such as dopaminergic agents, anticonvulsants, clonazepam or neuroleptic medications.

Prevalence of RLS, Peripheral Neuropathy, and Sleep Complaints

The "current prevalence" of RLS was 32%, whereas its "life prevalence" was 42%, and positive first-degree relative family history for RLS was 11%.

Neurological examination disclosed that 32% of patients did not have peripheral neuropathy, whereas 41% and 27% showed low and high likelihood of peripheral neuropathy respectively.

The prevalence of nocturnal legs cramps in the whole population was 51%, and that of frequent nocturnal legs cramps 25%.

The prevalence of insomnia was 48%, and of frequent insomnia was 43%. Eight % of the sample reported nocturnal eating episodes fulfilling the Sleep-Related Eating Disorder criteria, and 7% of the patients nocturnal smoking episodes during awakenings from nocturnal sleep.

According to the Berlin Questionnaire, 40% of patients were at high risk of having Obstructive Sleep Apnea Syndrome, whereas 41% and 19% at low and absent risk respectively.

The mean Morningness – Eveningness Questionnaire score was 58.17±10.11. According to the MEQ cronotypes, 58% of the patients had a morning circadian preference (7% extreme and 51% moderate), 37% were of intermediate type, and 5% showed an evening circadian preference (4% moderate and 1% extreme).

Finally, the mean ESS score was 6.34±3.83, and 16% of patients had a score > 10 consistent with excessive daytime sleepiness.

Prevalence and clinical features of pruritus

The Yosipovitch Questionnaire disclosed that 87 patients (54%) of the whole sample complained of pruritus at the moment of the observation, whereas 19 (12%) reported a previous history of itch.

Considering the whole group of 106 patients that experienced itch in their life, 25 subjects (24%) recalled the onset of this symptom during chronic renal failure, and 79 (75%) after the start of HD treatment. Moreover, 25 patients (24%) reported that pruritus was an intermittent symptom, being the day before, of, and after HD the one with worst discomfort for 9, 14, and 2 subjects respectively. Patients reported that pruritus could involve the following parts of the body: head (53%), back (73%), trunk (48%), abdomen (42%), legs (61%), arm (44%), and whole body (25%). Moreover, itch could be associated with additional symptoms such as pain (4%), sweating (5%), headache (2%), heat (5%), cold (2%), whereas 82% of the patients experienced pruritus without any ancillary discomfort.

Considering the 87 patients with current pruritus, 47% and 28% reported its daily and weekly occurrence respectively, whereas 14% and 10% experienced itch once every two weeks and once per month. Pruritus usually lasted few minutes in 45%, half an hour in 13%, and hours in 35% of the patients respectively. Fifty-one subjects (59%) reported a circadian fluctuation of the symptom, that could be worse in the morning (10%), during daytime (8%), in the evening (41%), at night (16%), and during periods of inactivity (32%). Pruritus had a negative impact on nocturnal sleep, as long as patients reported that it induced sleep onset insomnia (47%) and/or nocturnal awakenings (32), and 14% of the sufferers used to take hypnotics because of the afore-mentioned sleep complaints. Additionally, 37% of the patients were treated with specific antipruritic medications.

The 87 patients currently suffering from pruritus reported that the symptom was aggravated-alleviated by rest (21% - 10%), dry skin (62% - 1%), heat (39% - 2%), sweat (32% - 1%), clothing (48% - 2%), psychological stress (21% - 2%), food (14% - 11%), before HD (8% -

2%), activity (3% - 33%), sleep (25% - 17%), hot shower (15% - 29%), cold shower (1% - 39%), cold (3% - 30%), tiredness (25% - 2%), physical effort (3% - 22%), during HD (13% - 8%), and after HD (16% - 9%).

Pruritus had a negative impact on daily life, 36% of patients reported an effect on mood, 63% on behavior, 40% on concentration, 11% on appetite, 15% on libido, and 24% on general functioning.

Patients reported that pruritus could be described as tickling (37%), prickling (31%), insects on the skin (42%), stab (9%), pinch (33%), burning (43%). Moreover, 93% of patients stated that pruritus was a bothersome sensation, 86% annoying, 67% unbearable, and 43% worrying. At visual analogue scales, patients scored 1.7±2.2 for the current feeling of pruritus, 6.2±2.9 and 0.9±1.6 for the worst and mildest itch perceived, and 5.7±2.9 for the pruritus occurring after a mosquito bite (for comparison).

RLS was not associated with pruritus in our population. Nevertheless, we tested whether in the patients with pruritus the clinical features of this complaint differed in the patients with and without RLS. We did not find any association regarding the distribution of pruritus in the different body areas (e.g. involvement of the legs in 70% and 58% of patients with and without RLS respectively, p=0.334), nor considering the symptoms that usually accompanied pruritus (e.g. pain, heat) or the impact of pruritus on daytime activities and mood. Moreover, pruritus did not differ in terms of circadian occurrence in patients with and without RLS (e.g. 64% vs 55% reporting a circadian fluctuation of the symptom, p=0.371; and 42% vs 40% with worse pruritus at evening, p=0.847). However, pruritus had a worse impact on sleep quality in RLS vs non RLS sufferers because they more frequently reported to have difficulties in falling asleep (64% vs 47%, p=0.030), and more nocturnal awakenings (53% vs 25%, p=0.018), despite they did not use more hypnotics for these complaints (19% vs 11%, p=0.114). Finally, RLS patients more frequently described pruritus as a pinching (0.97±1.07 vs 0.51±1.00, p=0.013) and worrying (1.17±1.11 vs 0.75±1.11, p=0.041) sensation.

Association of current RLS to clinical data, sleep complaints, and laboratory parameters

Overall, RLS was significantly associated with female sex (49% versus 29%, p=0.012), positive first-degree relative family history of RLS (22% versus 6%, p=0.004), insomnia (67% versus 39%, p=0.001), frequent insomnia (59% versus 36%, p=0.007), increasing likelihood of peripheral neuropathy (18%, 41%, and 41% versus 39%, 41%, and 21% of patients with respectively absent, low, and high likelihood, p=0.006), and decreasing residual diuresis (8%, 28%, and 65% versus 34%, 13%, and 53% of patients with more than 500 mL/24/h, between 0 and 500 mL/24h, and absent residual diuresis, p=0.001; or 182±335 versus 388±500 mL/24h, p=0.029) (table 1).

RLS was not associated with other clinical features (e.g. age, BMI, circadian chronotype), including the load of comorbidity, the presence of diabetes, the history of kidney transplantation, and the cause of ESKD. Moreover, RLS was not associated to the HD features such as dialysis technique, vintage, dose, shift (not reported), and efficacy measured by means of the Urea Reduction Rate (URR) and single pool Kt/V (spKt/V).

Concerning sleep comorbidity, RLS was not associated with nocturnal leg cramps and pruritus, that could be regarded as potential mimics in this population. SRED (14% vs 6%) and nocturnal smoking (10% vs 5%) were more common among RLS sufferers, but without however reaching statistical significance. According to the Berlin Questionnaire and to the Epworth Sleepiness Scale, the risk of having obstructive sleep apnea and of being excessively sleepy during daytime were not associated to RLS as well.

Despite the fact that association of RLS with daytime sleepiness did not reach statistical significance, the IRLSS score was correlated with subjective sleepiness at the ESS (Pearson's r = 0.34, p=0.011), thus suggesting that in patients with RLS the evening discomfort in the legs could play a role in the determination of daytime sleepiness

Laboratory data in patients with and without RLS are reported in table 2. Indices of iron metabolism were not significantly associated with RLS, despite a trend for lower iron stores

in RLS patients in terms of ferritin, iron, and iron saturation. Similarly, indices of inflammation (C reactive protein, eritro-sedimentation rate, fibrinogen, white cells count) showed a trend of association with RLS, without reaching the statistical significance in the whole population. However, higher values of ESR (42±25 vs 26±18, p=0.004), fibrinogen (441±79 vs 386±84, p=0.004), C-reactive protein (1.9±4.5 vs 0.8±1.0, p>0.05), and white cells count (8±3 vs 7±3, p=0.023) in RLS versus non RLS patients were disclosed in the subgroup of patients belonging to the S.Orsola Dialysis Unit. Finally, lower values of ALT were associated to RLS.

Clinical predictors of RLS occurrence

The clinical features associated with RLS were tested as independent predictors in a logistic regression approach with RLS presence as dependent variable. According to the univariate analyses, the model included female sex, first-degree relative positive family history of RLS, increasing likelihood of peripheral neuropathy (low and high compared to the reference category absent likelihood), and decreasing residual diuresis (between 0 and 500 mL/24h and absent compared to the reference category over 500 mL/24h residual diuresis), and frequent insomnia.

The model showed a good fit with data according to the Hosmer and Lemeshow Test (p=0.463). Residual diuresis between 0 and 500 mL/24h and absent residual diuresis referred to the reference category of residual diuresis over 500mL/24h, high likelihood of peripheral neuropathy referred to the reference category of absent likelihood of peripheral neuropathy, and positive first-degree relative family history of RLS significantly predicted current RLS in the multivariate model (table 3). An interaction between female sex and positive first-degree relative family history of RLS was also analyzed, but it was not significant.

Clinical features of RLS in ESKD undergoing long-term HD treatment

As stated above, 68 patients ("life prevalence" of 42%) complained of RLS and 51 patients ("current prevalence" of 32%) had the RLS tetrad at the moment of the interview. Considering the 68 patients fulfilling the criteria for RLS, 71% and 29% reported an intermittent and continuous RLS course. Patients with current RLS had a mean IRLSS score of 15.3±7.4. Sixty-five % and 35% of them reported an intermittent and continuous RLS course.

Considering the whole RLS life-prevalence group, patients reported the onset of RLS symptoms at a mean age of 55.7±16.2 years. Forty-one % of cases were females, and 19% had a positive first-degree-relative family history with a clear preponderance of female relatives (11 out of 13 relatives, 85%). Forty-four patients (66%) recalled that the onset of RLS was associated with kidney disease (17% during chronic renal insufficiency, 49% after HD treatment), whereas 23 patients (34%) declared that RLS started before kidney disease diagnosis; 10 of the latter however reported RLS symptoms worsening after renal insufficiency. Moreover, 71% of patients described the RLS course as intermittent and 29% as continuous.

When compared with RLS patients without positive family history, RLS patients with positive first-degree relative family history showed increased female preponderance, and recalled symptoms onset more frequently in association with chronic renal insufficiency than with HD treatment (table 4).

Conversely, patients with ESKD-related onset of RLS (i.e. RLS arising either during chronic renal insufficiency or after HD) reported a later age at onset of RLS than patients with RLS arising before kidney disease, without differing significantly for positive family history of RLS or gender distribution. More in detail, when compared with patients with RLS arising before kidney disease, patients with RLS arising after HD treatment had a lower first-degree relative family history for RLS with comparable female preponderance, whereas patients with

RLS arising during chronic renal failure did not differ significantly for both family history and gender (table 5).

Finally, the 17 patients that did not present anymore RLS at the moment of the interview were less frequently women, and showed lower evidence of peripheral neuropathy than patients with current RLS, without differing significantly neither for family history nor for any other clinical or RLS feature, despite a non significant trend for a higher residual diuresis (table 4).

Table 1: Comparison of clinical data, sleep disorders and neurological examination of HD patients with and without RLS in the whole population.

	RLS (51 pts)	**No RLS (111 pts)**	
Clinical Data	Mean±SD	Mean±SD	p-value
Age	67.76±13.11	65.93±14.84	ns
Female Sex (%)	49%	29%	0.012
BMI	24.76±4.85	24.82±4.19	ns
Charlson-CI	4.92±2.15	4.77±1.88	ns
ESKD-CI	3.37±2.61	2.52±2.12	ns
Diabetes Mellitus (%)	31%	24%	ns
Transplantation (%)	10%	13%	ns
Dialysis Technique (diffusive, mixed)	71%-29%	87%-13%	ns
Dialysis Vintage (months)	44.09±42.96	37.84±45.46	ns
Dialysis sessions/week (number)	2.92±0.4	2.79±0.55	ns
Dialysis hours/session (hours)	3.96±0.26	3.99±0.22	ns
URR	66.3±10	66.88±10.1	ns
spKt/V	1.35±0.35	1.37±0.41	ns
Residual Diuresis (mL/24h)	182.35±335.23	388.32±500.92	0.029
Residual Diuresis Category (>500; 0-500; 0 mL/24h) (%)	8%-28%-65%	34%-13%-53%	0.001
Sleep Complaints			
RLS - first degree relative family history (%)	22%	6%	0.004
Nocturnal Legs Cramps (%)	61%	46%	ns
Frequent Nocturnal Legs Cramps (%)	28%	23%	ns
Insomnia (%)	67%	39%	0.001
Frequent Insomnia (%)	59%	36%	0.007
Sleep Related Eating Disorder (%)	14%	6%	ns
Nocturnal Smoking (%)	10%	5%	ns
Pruritus (%)	63%	50%	ns
Questionnaires			
Berlin Questionnaire (absent-low-high risk)	21%-33%-46%	18%-45%-37%	ns
Epworth Sleepiness Scale Score	6.98±4.21	6.04±3.62	ns
Epworth Sleepiness Scale Score>10 (%)	20%	14%	ns
Morningness-Eveningness Questionnaire	57.43±12.12	58.55±8.97	ns
MEQ chronotype (morning-intermediate-evening)	54%-41%-5%	60%-35%-5%	ns
Neurological Examination			
Polyneuropathy Likelihood	18%-41%-41%	39%-41%-21%	0.006

Table 2: Comparison of laboratory data of HD patients with and without RLS in the whole population.

	RLS now	**No RLS**	
	Mean±SD	Mean±SD	p-value
Hematocrit	33.67±3.83	33.36±4.37	ns
Hemoglobin (g/dL)	10.72±1.42	10.76±1.41	ns
MCV	*90.77±8.98*	*91.22±7.62*	ns
Iron	*46.51±22.82*	*50.15±27.56*	ns
Iron Saturation	*18.08±9.7*	*20.02±15.42*	ns
Ferritin	*256.61±343.32*	*271.07±417.37*	ns
TIBC	214.13±57.69	214.88±67.35	ns
UIBC	188.19±58.46	205.65±72.27	ns
C reactive protein	*1.84±3.6*	*1.05±1.84*	ns
Fibrinogen	*451.63±136.49*	*415.06±109.57*	ns
ESR	*44.16±24.71*	*25.74±17.77*	*0.001
White Cells	*7.43±2.4*	*6.72±2.38*	ns
Platelet	287.33±124.89	258.58±90.33	ns
Albumin	3.76±0.42	3.84±0.41	ns
Proteins	6.6±0.47	6.65±0.83	ns
Creatinin	9.58±2.66	9.4±2.58	ns
Azotemia	164.6±40.73	161.6±41.37	ns
Uric Acid	6.59±1.6	8.33±17.81	ns
Intact PTH	331.43±234.01	324.69±278.56	ns
Bilirubin	0.28±0.06	0.31±0.11	ns
ALT	12.11±9.54	17.14±15.29	0.025
AST	13.04±6.03	16.1±10.28	ns
FosfAlc	251.51±101.35	223.38±96.74	ns
gGT	30.31±30.01	33.74±33.32	ns
Amilasemia	107.74±62.76	108.13±48.48	ns
Cholesterol	161.43±49.3	163.95±42.8	ns
HDL	37.18±11.8	37±11.46	ns
Triglicerids	209.02±146.22	182.23±82.21	ns
Glucose	116.72±41.42	111.28±51.42	ns
Calcium	8.83±0.87	8.75±0.89	ns
Potassium	5.57±0.69	5.49±0.9	ns
Sodium	138.94±3.6	139.37±3.89	ns
Phosphorus	5.25±1.51	5.54±1.82	Ns

*available only for patients belonging to the S.Orsola-Malpighi Dialysis Unit.

Table 3: Multivariate logistic regression model for current RLS in the whole population.

Variables	**P-value**	**Odd Ratio**	**95% Confidence Interval**
First-degree relative family history for RLS	0.030	3.567	1.128 - 11.278
Female Sex	0.058	2.178	0.974 - 4.873
Residual diuresis > 500mL/24h (reference)	0.009		
Residual diuresis between 0 and 500mL/24h	0.002	8.115	2.101 - 31.336
Residual diuresis = 0 mL/24h	0.012	4.621	1.400 - 15.250
Absent likelihood of peripheral neuropathy (reference)	0.045		
Low likelihood of peripheral neuropathy	0.183	1.971	0.727 - 5.348
High likelihood of peripheral neuropathy	0.013	3.728	1.315 - 10.571
Frequent insomnia	0.122	1.822	0.852 - 3.899
Constant	0.000	0.029	

Table 4: Clinical features of RLS patients subgroups: patients with and without positive family history, and patients with and without current RLS.

	Family history +	**Family history -**	p	**Current RLS**	**Previous RLS**	p
Female Sex	77%	33%	0.004	49%	18%	0.023
First-degree relative family history for RLS	100%	0%	/	22%	12%	ns
RLS onset: before Kidney Disease	46%	32%	ns	36%	29%	ns
RLS onset: Chronic Renal Failure	39%	13%	0.031	18%	18%	ns
RLS onset: Hemodialysis Treatment	15%	57%	0.007	47%	53%	ns
Age at onset of RLS	50±11	57±16	ns	56±16	55±16	ns
Peripheral Neuropathy (absent, low, high)	23%;54%;23%	25%;40%;35%	ns	18%;41%;41%	47%;47%;6%	0.009
Residual Diuresis category (>500; 0-500; 0 mL/24h)	16%;15%;69%	11%;24%;65%	ns	8%;27%;65%	23%;6%;71%	[0.066]

Table 5: Clinical features of RLS patients subgroups: patients with onset of RLS before kidney disease (non uremic), during chronic renal failure, and after hemodialysis treatment start

	Female Sex	***First-degree relative family history***	***Age at onset of RLS***	***Peripheral Neuropathy likelihood (absent, low, high)***	***Residual Diuresis category (>500; 0-500; 0 mL/24h)***
A - non uremic onset	44%	26%	46±18	26%;48%;26%	9%;22%;69%
B - uremic onset	41%	16%	60±14	25%;41%;34%	14%;20%;66%
B1 - CRF onset	67%	42%	59±10	23%;62%;15%	23%;31%;46%
B2 - HD onset	33%	6%	58±15	29%;31%;40%	14%;14%;72%
A vs B, p	ns	ns	0.007	ns	ns
A vs B1, p	ns	ns	0.03	ns	ns
A vs B2, p	ns	0.035	0.013	ns	ns

Impact of RLS on the HD procedure

Relation between RLS and HD procedure

Considering the relation between RLS symptoms and HD treatment, 44 patients (66%) did not report any change of RLS symptoms related to day of HD (42% intermittent RLS, 24% continuous RLS), whereas 23 patients (34%) declared an influence of HD day on RLS (12% and 22% had worse symptoms in the evening before and of HD respectively).

Thirty-five patients (52%) complained of RLS during HD sessions, as a rare (23%) or frequent (28%) discomfort, that forced at least once to early dialysis discontinuation 11 patients (16%).

The mean start time for RLS discomfort was at 18.7±6.4, but 15 (23%) subjects reported significant differences of RLS timing in the days with (mean start time at 16.5±5.8) or without (mean start time at 19.7±3.3) HD, suggesting an anticipation of RLS symptoms [of about 1.4±5.3 hour] related to dialysis treatment in this patient subgroup.

Impact of RLS on fatigue, quality of life, and mood

Patients with and without current RLS were contrasted for self reported scores at the fatigue severity scale (FSS), SF-36, and BDI-2.

Patients with current RLS did not differ significantly for the FSS score (42.1±15.3 vs 36.1±17.4, $p>0.05$), but showed lower quality of life scores at the SF-36 (77.2±20.7 vs 91.2±22.5, $p=0.002$), and higher depression scores at the BDI-2 (21.4±14.2 vs 14.7±12.5, $p=0.006$) than patients without RLS at the moment of the clinical assessment. Moreover, all the partial subscores of the SF-36, but not the mental health one, showed lower scores in patients with current RLS compared to patients without RLS (table 6).

Despite RLS patients did not reach statistical significance for their daytime fatigue (FSS) as well as for their daytime sleepiness (ESS) in comparison with patients without RLS, these scales were significantly and positively correlated with the IRLSS score, thus suggesting that in patients with RLS the intensity of the evening leg discomfort negatively influenced both fatigue and sleepiness during daytime (table 7). Moreover, all the partial subscores of SF-36, but not the mental health one, the global SF-36 score, and the BDI-2 score were positively correlated with the IRLSS score, thus confirming the detrimental effect of RLS on mood and all measured dimensions of the quality of life, with the single exception of the mental health area (table 7).

Table 6: Comparison of daytime symptoms, quality of life and mood between patients with and without current RLS.

	RLS	**Non RLS**	
	Mean±SD	*Mean±SD*	*Mann Whitney*
ESS score	6,98±4,21	6,04±3,62	0,234
FSS score	42,05±15,32	36,12±17,44	0,094
SF-36: PF	33,41±24,35	48,13±31,51	0,015
SF-36: RF	11,18±23,27	31,88±38,97	0,008
SF-36: BP	43,54±28,8	60,30±31,39	0,006
SF-36: GH	25,49±17,94	35,25±22,91	0,034
SF-36: VT	37,44±23,38	48,38±24,9	0,019
SF-36: SF	43,29±27,96	58,75±26,34	0,005
SF-36: RE	28,71±37,76	51,67±43,38	0,007
SF-36: MH	58,49±25,4	64,6±22,33	0,210
SF-36 Global score	77,21±20,72	91,17±22,51	0,002
BDI-II score	21,35±14,22	14,70±12,55	0,006

Table legend: physical function (PF), role limitations related to physical problems (RF), bodily pain (BP), general health perception (GH), vitality (VT), social functioning (SF), role limitations due to emotional problems (RE), and mental health (MH).

Table 7: Correlations between RLS severity at the IRLSS and daytime symptoms, quality of life and mood in patients with current RLS.

	Coefficient	*P-value*
ESS score	0,34	0,011
FSS score	0,42	0,002
SF-36: PF	-0,38	0,004
SF-36: RF	-0,40	0,002
SF-36: BP	-0,50	<0,001
SF-36: GH	-0,33	0,014
SF-36: VT	-0,41	0,002
SF-36: SF	-0,38	0,004
SF-36: RE	-0,35	0,007
SF-36: MH	-0,19	0,160
SF-36 Global score	-0,43	0,001
BDI-II score	0,29	0,026

Table legend: physical function (PF), role limitations related to physical problems (RF), bodily pain (BP), general health perception (GH), vitality (VT), social functioning (SF), role limitations due to emotional problems (RE), and mental health (MH).

Impact of RLS on survival and occurrence of new cardiovascular events at 18 months follow-up

We prospectively evaluated the occurrence of new cardiovascular events (myocardial infarction, cerebral stroke, and peripheral artery occlusion, clustered together as CVE) and the survival at 18 months follow-up in the cohort of 100 patients belonging to the S.Orsola Malpighi Dialysis Unit. This group of patients was chosen for this observational study because it was the first we evaluated at baseline and is currently followed by means of a internet-based database by our colleagues of the S.Orsola Malpighi hospital. Moreover, the prevalence of current RLS in this subgroup of patients was 31%, a value perfectly comparable to that of the whole population (32%). Similarly the factors associated to RLS in this subgroup of patients were comparable to the findings we obtained from the whole population and will be briefly presented for the purposes of this thesis section. These results have already been published in a peer-reviewed journal (La Manna, 2010).

RLS: associated clinical and biochemical parameters

The prevalence of RLS in the subgroup of the 100 patients enrolled in the study was 31%, with continuous and intermittent course in 30% and 70% of cases respectively.

Patients with RLS were more frequently women (52% vs 30%, p=0.042), with reduced/absent residual diuresis (3%, 23%, and 74% vs 26%, 3%, and 71% with >500, 0-500, and 0 mL/24h) than patients without RLS, in the absence of further differences regarding clinical (e.g. BMI, age, comorbidity load) or HD (vintage, technique, dose, and efficacy) features. Moreover, patients with RLS showed biochemical evidence of chronic inflammation disclosed by higher white cells count (7.8±2.5 vs 6.7±2.6 $10^3/mm^3$, p=0.026), eritrosedimentation rate (44.2±24.7 vs 25.7±17.8 mm/h, p=0.001), fibrinogen (444.6±76.0 vs 386.2±83.6 mg/dL, p=0.001), a

trend for higher C-reactive protein (1.2±1.2 vs 0.8±0.8 mg/dL, p=0.19), and of malnutrition as measured by lower albumin levels (3.6±0.4 vs 3.8±0.5 mg/dL, p=0.039).

RLS and outcomes

At baseline, 71% of patients with RLS versus 55% of patients without RLS (p>0.05) already had a previous CVE. The mean number of CVE was 1.48±1.29 and 1.10±1.27 in RLS and non-RLS patients respectively (p=0.127).

The occurrence of new cardiovascular events and the mortality during the 18 months of follow-up were 47% and 20% respectively.

At the 18 months follow-up, more patients with RLS had experienced a new CVE than patients without RLS (64% vs 39%, p=0.019) (figure 1A). This finding remained unchanged at multivariate analysis (multivariate Cox model) after including all factors associated with new CVE occurrence at univariate analyses (table 8A). The mean number of new CVE was 0.68±0.65 and 0.52±0.80 (p=0.101), and the total number of CVE was 2.16±1.73 and 1.62±1.81 (p=0.101) in patients with and without RLS respectively.

Further partitioning our RLS population in subgroups of patients with intermittent (I-RLS) and continuous (C-RLS) course, we found that C-RLS was more strongly associated than I-RLS to the occurrence of new CVE (88.9% and 57.1% in C-RLS and I-RLS respectively) (figure 1B). Accordingly, the multivariate analysis confirmed that C-RLS vs I-RLS patients had an higher odd ratio for new CVE, despite these findings did not reach statistical significance (table 8B).

RLS was also associated with higher mortality: 32.3% versus 14.5% of patients with and without RLS deceased during the observation period (p=0.014) (figure 2A). Figure 3 depicts the Kaplan – Meier curves of the two groups of patients highlighting that patients without RLS constantly showed a higher survival rate (log-rank test p=0.057). The multivariate analysis (Cox model) taking into account the confounding effects of other clinical and

demographic factors confirmed the independent association of RLS with higher mortality with an hazard ratio of 3.28 (95% Confidence Interval, CI=1.08-9.93) (table 9A).

Further partitioning our RLS population in subgroups of patients with intermittent and continuous course disclosed that mortality was significantly (p=0.014) higher in patients with C-RLS (55.6%) versus I-RLS (23.8) (figure 2B). At the multivariate analysis (Cox model), the dissection of C-RLS and I-RLS resulted in a further increase of the association between mortality and C-RLS with an hazard ration of 6.29 (95% CI=1.74-22.79) (table 9B).

Figure 1: Prevalence of new cardiovascular events according to the overall presence of RLS (A) and the presence of RLS with intermittent (I-RLS) vs continuous (C-RLS) course (B).

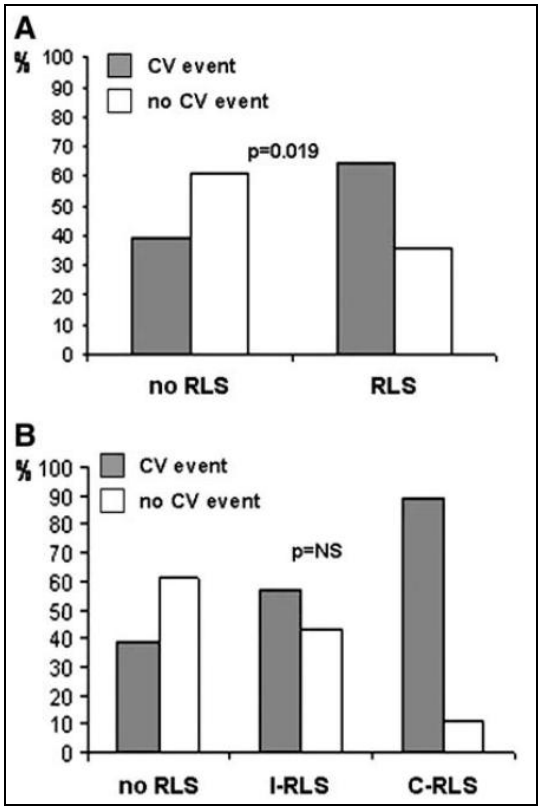

Table 8: multivariate Cox model for new cardiovascular events.

	OR (95%CI)	**p-value**
Model A		
Age	1.04 (1.01 - 1.07)	**0.014**
Male gender	1.15 (0.57 - 2.30)	0.705
Charlson Comorbidity Index	1.08 (0.90 - 1.30)	0.418
Proteinemia	0.70 (0.30 - 1.65)	0.417
Residual diuresis (> 0 vs. 0)	0.66 (0.28 - 1.58)	0.349
CRP	1.16 (0.75 - 1.80)	0.504
Albumin	1.20 (0.37 - 3.91)	0.768
HD vintage	1.00 (0.99 - 1.01)	0.451
HD hours/session	1.21 (0.05 - 30.34)	0.908
RLS vs. No RLS	2.01 (0.98 - 4.12)	**0.056**
Model B		
Age	1.04 (1.00 - 1.07)	**0.025**
Male gender	1.13 (0.56 - 2.28)	0.732
Charlson Comorbidity Index	1.09 (0.90 - 1.31)	0.386
Proteinemia	0.65 (0.26 - 1.64)	0.363
Residual diuresis (> 0 vs. 0)	0.66 (0.27 - 1.58)	0.349
CRP	1.19 (0.76 - 1.86)	0.457
Albumin	1.22 (0.37 - 4.01)	0.743
HD vintage	1.00 (0.99 - 1.01)	0.405
HD hours/session	1.10 (0.04 - 27.99)	0.956
I-RLS vs. No RLS	1.83 (0.77 - 4.36)	0.173
C-RLS vs. No RLS	2.36 (0.83 - 6.71)	0.108

Figure 2: All-cause mortality according to the overall presence of RLS (A) and the presence of RLS with intermittent (I-RLS) or continuous (C-RLS) course (B).

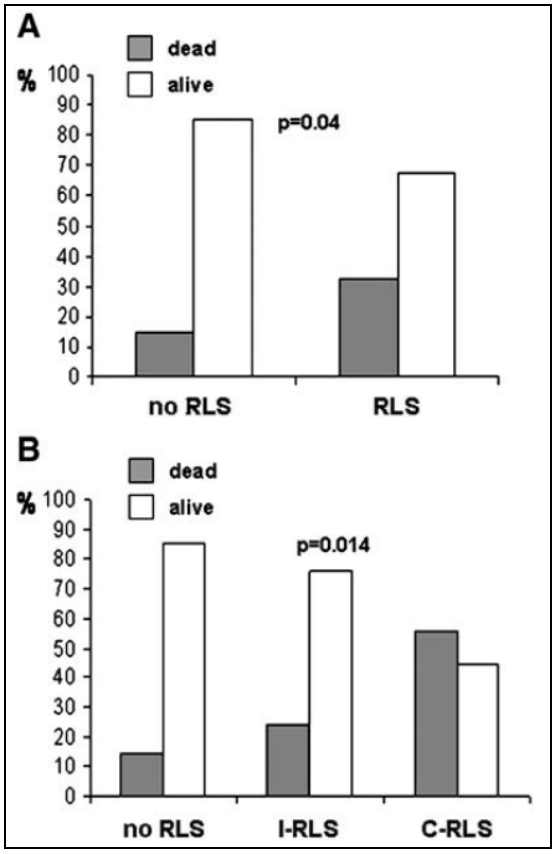

Figure 3: Kaplan-Meier estimates of all-cause mortality at 18 months for patients with and without RLS.

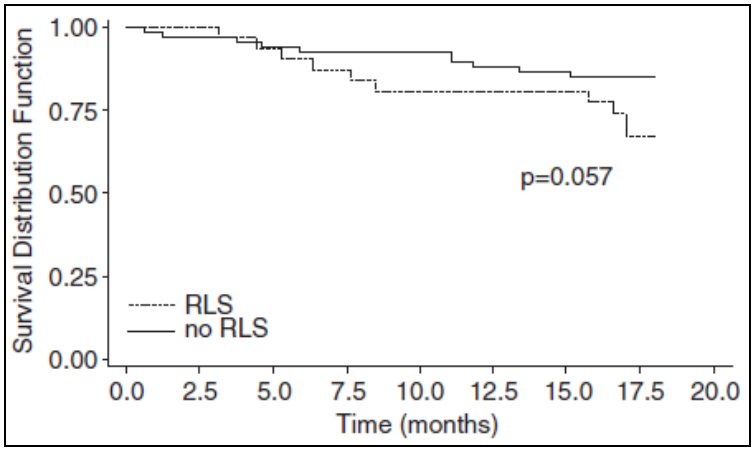

Table 9: Results from multivariate Cox analysis for all-cause mortality.

	HR (95%CI)	**p-value**
Model A		
Age	1.03 (0.99 - 1.08)	0.185
Male gender	1.79 (0.55 - 5.87)	0.337
BMI	0.80 (0.66 - 0.97)	**0.024**
Charlson Comorbidity Index	1.33 (1.11 - 1.61)	**0.003**
Albumin	0.40 (0.11 - 1.43)	0.157
Residual diuresis (>0 vs. 0)	0.53 (0.06 - 4.78)	0.574
RLS vs. No RLS	3.28 (1.08 - 9.93)	**0.035**
Model B		
Age	1.02 (0.98 - 1.07)	0.349
Gender male	1.45 (0.43 - 4.82)	0.549
BMI	0.8 (0.66 - 0.97)	**0.025**
Charlson Comorbidity Index	1.35 (1.13 - 1.63)	**0.001**
Albumin	0.29 (0.07 - 1.15)	0.078
Residual diuresis (>0 vs. 0)	0.55 (0.06 - 5.03)	0.598
I-RLS vs. No RLS	1.87 (0.47 - 7.40)	0.375
C-RLS vs. No RLS	6.29 (1.74 - 22.79)	**0.005**

Chapter 4: Discussion

The aim of the present research project was the full characterization of RLS in a homogeneous population of patients with ESKD undergoing stable HD in order to answer the following research questions: 1, what is the prevalence of clear-cut RLS, and what is its relation with kidney disease?; 2, which are the clinical determinants of RLS, taking into account clinical features, laboratory data, kidney – related factors, neurological evidence of peripheral neuropathy, patients' comorbidity, and genetic background (i.e. first-degree family history as surrogate marker of a genetically determined predisposition)?; 3, what is the impact of RLS on nocturnal sleep, daytime sleepiness, fatigue, quality of life, and mood?; and 4, what is the impact of RLS on the occurrence of new cardiovascular events and on survival at 18 months follow-up?

In this section of the dissertation, the above mentioned points will be discussed in relation with the available evidences from the scientific literature. The literature was screened using Pubmed and applying different combinations of the following keywords: Restless Legs Syndrome, Kidney, Renal, End Stage Kidney Disease, Hemodialysis, Dialysis, Secondary, Laboratory. All scientific articles on RLS in patients with kidney disease were reviewed, and particular attention was devoted to check the following items: number of patients evaluated, criteria used to define RLS, assessment strategy for RLS (e.g. self-administered questionnaire vs clinical interview), the associations found between RLS and different clinical or laboratory data, and, notably, the associations that were not found/replicated with clinical and laboratory data.

Prevalence of RLS*: *assessment strategy and role of mimics

The prevalence of RLS in our study was assessed by means of a semi-structured clinical interview performed by a neurologist expert in sleep medicine as certified by the Italian Association of Sleep Medicine.

We believe that this approach is a key advantage of our study compared with several previous reports, as mirrored by the comparable prevalence we observed in the two HD units (31% vs 32%, not significant, data not shown). Accordingly, a previous study performed by Cirignotta et al compared RLS prevalence assessed by means of self-administered questionnaires using the criteria established in 1995 (Walters, 1995) vs a clinical interview performed by neurologists expert in sleep medicine, and disclosed that in the clinical setting of ESKD patients undergoing chronic HD the two approaches could lead to different results (27% and 33% for questionnaires and clinician respectively) (Cirignotta, 2002). Interestingly, the authors found that patients with a false positive RLS diagnosis at the questionnaire frequently complained of symptoms at lower limbs and showed abnormal neurological examination, thus strongly suggesting the co-occurrence of a peripheral neuropathy that could not be further demonstrated in the absence of EMG studies of other systematic assessments. Conversely, false negative RLS patients at the questionnaire were patients with clear-cut RLS, but mild symptoms (Cirignotta, 2002).

Recently, great emphasis has been devoted in pinpointing the potential role of other confounders that may fulfil the four RLS criteria in the general population and be regarded as "RLS mimics" (Hening, 2009). Therefore, great caution should be devoted in the interpretation of self-administered questionnaires and a careful assessment of other potential mimics is recommended in order to establish which patients really have RLS as a pure condition, or eventually associated with other comorbidities. In our setting, we systematically assessed the concurrent presence of potential mimics such as nocturnal legs cramps, pruritus, other sleep complaints, and to evaluate the potential occurrence of peripheral neuropathy.

Indeed, leg cramps are highly prevalent in ESKD patients for electrolyte imbalance, as well as pruritus, the latter frequently involving the legs and being more bothersome in the evening/night (Zucker, 2003).

Moreover, the criteria to identify patients with RLS are continuously being developed and updated (Ekbom, 1945; Walters, 1995; Allen, 2003), making therefore the comparison between different studies intrinsically different not only for the assessment method, but also for the syndrome definition by itself.

Table 1 summarizes the available studies on RLS in patients with ESKD, reporting the year of publication, the criteria applied, the assessment strategy, the number of patients evaluated, and, finally, the prevalence of RLS in patients with ESKD. Despite the fact that the available literature showed a wide range of estimation of RLS prevalence ranging from 8% to 60%, all authors support the concept that RLS is more prevalent among patients with ESKD than among the general population. Accordingly, Winkelman et al also supported the pathogenetic role of the ESKD condition comparing the prevalence of RLS in renal (20%) versus chronic heart failure (6%) patients (Winkelman, 1996). However, the different findings are clearly influenced by the different criteria applied and by the use of different assessment strategies. Disregarding these important differences, the mean reported prevalence of RLS in the literature is 31%, a value that is strikingly in line with our finding of 32%.

Moreover, according to Hening et al (Hening, 2009), we systematically evaluated potential mimics, and in our approach we clearly distinguished common sleep-related and clinical comorbidities in order to test potential associations with RLS. In regard to the "mimics" concern, our clinical experience with ESKD patients in the two HD units and with the clinical setting of sleep medicine in the Outpatient Clinic for Sleep Disorders of the Department of Neurological Sciences of the University of Bologna suggested some considerations. First, RLS can be clearly identified in ESKD patients and distinguished from other confounding symptoms such as leg cramps occurring during daytime, mainly after the HD procedure, or

during night-time, as well as from distal paraesthesias or other potential symptoms suggesting a peripheral neuropathy, the latter having a prevalence ranging from 60% to 90% (Krishnan, 2009). Second, RLS is not a primary complaint for most of ESKD patients that suffer from a high load of medical comorbidities and undergo very demanding procedures, but RLS can be clearly identified on a clinical basis when appropriately investigated.

Table 1: overview of the studies on RLS prevalence in patients with ESKD.

Year	Author - Journal	Population	Criteria	Method	Prevalence
1991	Roger - Lancet	55 PD-HD	N.A.	Interview	40%
1995	Walker - A.J.K.D.	54 HD	I.C.S.D.1990	Questionnaire	57%
1996	Winkelman - A.J.K.D.	222 HD	Walters 1995	Questionnaire	20%
1998	Collado-Seidel - A.J.K.D.	136 HD	Walters 1995	Interview	23%
1998	Virga - I.J.A.O.	73 HD	N.A.	Questionnaire	8%
2000	Huiqi - Nephron	38 HD	Walters 1995	?	34%
2002	Cirignotta - A.J.K.D.	127 HD	Walters 1995	Quest. Vs Int.	27% vs 33%
2003	Takaki - A.J.K.D.	490 HD	Walters 1995	Questionnaire	12%
2004	Unruh - A.J.K.D.	894 HD	Single question (?)	Questionnaire	15%
2004	Rijsman - Nephrology	48 HD	Allen 2003	Interview	58%
2005	Mucsi - N.D.T.	333 HD	Walters 1995	Questionnaire	14%
2005	Siddiqui - N.C.P.	227 HD	Walters 1995	Questionnaire	46%
2006	Kawauchi - Clin.Nephrol.	228 HD	Walters 1995	Questionnaire	23%
2006	Merlino - N.D.T.	883 HD	Walters 1995	Questionnaire	18%
2006	Unruh - C.J.A.S.N.	909 HD-PD	?	Questionnaire	?
2007	Telarovic - Eur.Neurol.	82 HD	Allen 2003	Interview	60%
2007	Bastos - R.A.M.B.	210 HD	I.C.S.D.1990	Questionnaire	48%
2008	Hsu - Artif.Org.	150 HD	2 questions (?)	Questionnaire	23%
2008	Kim - J.Clin.Neurol.	164 HD	Walters 1995	Interview	28%
2009	Al-Jahdali -S.J.K.D.T.	227 HD	Allen 2003	Questionnaire	50%
2009	Szentkiralyi – J.Psych.Res.	161 HD	Walters 1995	Questionnaire	11%
2010	Araujo - S.M.	400 HD	Allen 2003	Questionnaire	22%
2010	Sabry - S.J.K.D.T.	88 HD	Walters 1995	Questionnaire	42%
2010	La Manna - N.D.T.	100 HD	Allen 2003	Interview	31%
2011	Salman - S.J.K.D.T.	123 HD	Walters 1995	Questionnaire	20%

Associations between RLS and other clinical or laboratory factors

Previous studies on RLS in patients with ESKD undergoing HD disclosed different associations with clinical and laboratory features, as summarized in tables 2 and 3. Indeed, even if the complex clinical condition of ESKD shares many of the factors that are by themselves well-established causes of secondary RLS, such as anemia, iron depletion and peripheral neuropathy, the studies already performed provide sometimes conflicting results as to the clinical determinants of RLS, offering thus insufficient insights into disease pathophysiology. Female sex, age, comorbidity load, diabetes mellitus, peripheral neuropathy, iron depletion, anemia, calcium and parathormone levels, and HD features (e.g. vintage, shift, and dose) were associated with RLS in ESKD patients undergoing HD in some studies, but were repeatedly not replicated in others (tables 2 and 3).

Gender and family history: the role of genetic factors

In our work, we disclosed a very strong association with female sex. This finding has been consistently reported in five previous reports, albeit not replicated in other eight studies that evaluated the gender influence (table 2). Moreover, the association between RLS and female gender is well-known in the context of idiopathic RLS (AASM, 2005), and pinpoints the potential role of genetic and hormonal factors.

At least the other following two personal finding are of interest from a pathophysiological standpoint and could be discussed together with the role of sex: first, the association of current RLS with positive first-degree relative family history of RLS; second, at least one third of our RLS sufferers recalled symptoms' onset before any (known) evidence of kidney disease. Surprisingly, these two clinical factors had never been investigated in the context of uremic RLS. Taken together, these results support the theoretical concept that along the course of kidney failure (i.e. from chronic renal disease to ESKD) some environmental factors

may trigger the expression of a pre-existent genetic background common to idiopathic RLS at least in a part of subjects thus increasing the number of subjects suffering from RLS.

Several studies have shown the predisposing role of genetic factors for idiopathic RLS, given its high rate of positive familial history, the identification of several loci in families with recurrence of RLS (Winkelmann, 2007), and the association with genetic variants in non-familial RLS patients (Winkelmann, 2007b; Stefansson, 2007). In these genetic studies the combined analysis of the possible risk alleles showed however a limited value for individual risk prediction, pointing to the mutual contribution of genetic and non genetic factors to the risk of RLS (Schormair, 2008).

On the other hand, recent data disclosed an increased prevalence of RLS also during chronic renal failure not requiring HD (Merlino, 2010), similarly to our finding of several patients recalling RLS onset before the start of HD treatment, either before renal failure or after kidney disease diagnosis. Indeed, Merlino et al found that RLS was more common in non-dialyzed patients with chronic renal failure (11%) compared to controls (3%), and also disclosed an association with female sex and biochemical evidence of iron deficiency. The authors also investigated familial recurrence of RLS only in affected individuals, disclosing that a significantly larger part of controls (50%) compared to chronic renal failure patients (13%) reported a positive family history. The reported family history in these RLS subjects with chronic kidney disease (Merlino, 2010), and with ESKD requiring HD (15%) (Araujo, 2010) are comparable to our finding of 22%. However, in the absence of information on family history in subjects without RLS, the authors could not infer on the role of this genetic factor in the determination of RLS in their studies (Merlino, 2010; Araujo, 2010). Another recent study evaluating the prevalence of RLS in a population of older patients acutely admitted to hospital disclosed that RLS was independently associated with biochemical evidence of iron deficiency and of chronic kidney disease not requiring HD, as demonstrated by lower levels of ferritin and of estimated glomerular filtration (Quinn, 2011).

Clinical and demographic influences

As summarized in table 2, RLS has been patchy associated with higher (Unruh, 2004) and lower (Kawauchi, 2006) age, but most studies did not replicate this finding. Accordingly, we did not found any significant trend for age differences between RLS and non-RLS patients. Given the well-established increase of idiopathic RLS prevalence in different age classes in the general population (AASM, 2005), the absence of a clear age effect in our study further corroborates the strong role of the ESKD condition in the determination of RLS.

Another important finding disclosed by some previous studies was the association of RLS with diabetes and/or higher comorbidity load in ESKD patients. We emphasize that the comorbidity assessment was only rarely performed by means of a structured comorbidity index, therefore the literature data were difficult to compare to our study (table 2). Moreover, RLS was found to be more frequent in diabetic patients, and to be associated with evidence of peripheral neuropathy, versus normal controls also out of the context of ESKD (Merlino, 2007), thus adding importance to the need for differentiating the impact of ESKD from that of diabetes itself. In our study, we found a non-significant trend for higher comorbidity (and diabetes), that is therefore in line with the current available data. However, the absence of any significant result pinpoints that the ESKD condition could lead to the high prevalence of RLS independently from other clinical comorbidities.

Kidney-related factors

Previous studies disclosed an association of RLS with higher HD dose measured by the number of HD sessions per week (Huiqi, 2000), with higher HD vintage measured by the time elapsed from the beginning of HD treatment (Siddiqui, 2005), with lower age at HD treatment start (Kawauchi, 2006), and with the afternoon shift (Hsu, 2008; Al-Jahdali, 2009). Conversely, several authors failed to replicate the above findings, and any kidney-related factor was intrinsically associated with RLS in ESKD patients (Winkeman, 1996; Collado-

Seidel 1998; Takaki, 2003; Mucsi, 2005; Merlino, 2006; Bastos, 2997; Kim, 2008; Araujo, 2010; Sabry, 2010; Salman, 2011).

Our study systematically investigated the cause of ESKD, the HD techniques used (including membranes), the HD efficacy (URR, spKt/V), the HD dose, vintage, shift, and, finally, the residual diuresis of ESKD patients. Only a lower residual urine output was significantly associated with RLS in our population, a finding never reported before to our knowledge in this setting, whereas HD shift was a variable intrinsically biased by the clinical practice of our colleagues that used to chose the HD timing on the basis of other clinical considerations.

The residual urine output is important in ESKD patients, because it is an indirect parameter measuring the residual renal function. When considering the purification role of kidney function, we know that the so-called "big" molecules can be easily filtered by using different membranes during the HD procedure, whereas the so-called "small" and "middle" molecules tend to remain into the blood circulation unless captured by specific absorbents. Accordingly, the residual renal function provides an additional purification targeted on these substances that otherwise can accumulate into the body, even if by itself is insufficient to protect against uremia as demonstrated by the need for HD. Moreover, the deterioration of residual renal function in ESKD patients undergoing HD may worsen anemia, inflammation and malnutrition contributing to the poor prognosis of this patients population (Chandna, 2004).

To date, the main evidences connecting kidney function to RLS are of two different orders: first, RLS is more frequent in patients with ESKD (table 1) and chronic kidney failure (Merlino, 2010; Quinn, 2011) compared to the general population; second and most important, several studies proved an amelioration of RLS up to its disappearance after efficacious kidney transplantation (Winkelmann, 2002; Molnar, 2005). Accordingly, the amount of available data indirectly connects kidney function to RLS in ESKD patients, and our finding of lower residual renal function in RLS sufferers should be regarded as the first intrinsic link between kidney function and RLS occurrence. Accordingly, given the described

association between declining renal function after kidney transplantation and increasing prevalence of RLS (Molnar, 2005), we may speculate that renal function directly affects RLS occurrence: its decrease leads to a wide range of kidney insufficiency from chronic renal failure (Merlino, 2010; Quinn, 2011) to ESKD requiring HD (table 1), conditions characterized by an increased occurrence of RLS; its "restitutio ad integrum" by means of kidney transplantation reverses the RLS symptoms in a few days (Winkelmann, 2002); and its progressive worsening during graft failure leads to the re-occurrence of RLS (Molnar, 2005). Intriguingly, RLS was found to be a significant risk factor for mortality in kidney transplanted patients (Molnar, 2007), and the question whether RLS may worsen by itself the outcome of this patients' population or may reflect a worse renal condition remains actually unanswered. Therefore, RLS in the course of kidney failure may be regarded as a "functional" disturbance closely related to blood purification, and to the potential accumulation of "toxic" substances that however have not yet been identified and cannot be reliably estimated by conventional HD efficacy measures.

Peripheral neuropathy

In our study we systematically evaluated the presence of symptoms and signs in order to establish the estimated likelihood of distal symmetric polyneuropathy, assessed according to the consensus criteria for clinical and epidemiological research studies (England, 2005). Previous studies on uremic RLS failed in applying a systematic stratification of patients for the peripheral neuropathy risk, thus reporting some negative data on the relation between RLS and nerve conduction velocity assessed by single nerve electroneurography (Winkelman, 1996; Rijsman, 2004) or clinical records with evidence of a documented (without any further information) peripheral neuropathy (Araujo, 2010). Moreover, the potential mimicking role of signs at neurological examination highly suggesting a peripheral neuropathy has been proved,

thus highlighting the need for a systematic investigation to correctly interpret RLS prevalence results (Cirignotta, 2002).

We found a clear association between clinical evidence of increasing risk for a peripheral neuropathy and RLS in ESKD patients. This result is in line with the early descriptions of uremic RLS (Callaghan, 1966), with studies of the association between RLS and peripheral neuropathy (secondary RLS) (Rutkove, 1996; Salvi, 1990; Ondo, 1996; Gemignani, 2006), and with other medical conditions such as diabetes mellitus where peripheral neuropathy is intrinsic to the disease comparably to ESKD (Merlino, 2007), as well as with subclinical evidence disclosed by nerve or skin biopsy in otherwise idiopathic RLS (Iannacone, 1995; Polydefkis, 2000). Taken together, these data indicate that RLS may be regarded as a symptom of a peripheral neuropathy, but also that a peripheral neuropathic condition may be involved in the pathogenesis of RLS either for the altered afferent signaling or for the long-term rearrangements occurring at the central nervous system level.

Considering the ESKD condition, the development of an uremic neuropathy is exceedingly common with prevalence rates reaching 60 to 90% in subjects undergoing HD (Krishnan, 2009). Uremic neuropathy generally presents as length – dependent polyneuropathy, in which the earliest clinical features reflect the involvement of large, myelinated sensory fibers, causing paraesthesias and numbness (Krishnan, 2007). In this context, we must underline that a common cause of ESKD is diabetes, another clinical condition that may significantly increase the burden of peripheral neuropathy in this patients' population.

Different theoretical concepts may underline the relation between ESKD and peripheral neuropathy occurrence. First, uremia is characterized by the retention of solutes that are toxic in high concentrations such as urea, creatinine, parathyroid hormone, myoinositol, beta-2 microglobulin, and may mediate the occurrence of neurological complications acting as "neurotoxins". Accordingly, previous studies demonstrated the following results: 1, conduction slowing in clinically unaffected nerve segments; 2, a correlation between these

neurophysiological abnormalities and the severity of renal impairment; and 3, the rapid improvement of clinical symptoms and nerve conduction parameters after few days from renal transplantation (Nielsen, 1973; Nielsen, 1974, 1974b; Oh, 1978;). Despite several authors suggesting that the so-called "middle-molecules" may be responsible for the development of these neurological dysfunctions, little evidence exists demonstrating that such substances are actually neurotoxic (Vanholder, 1994). Second, other authors hypothesized that in the ESKD condition the biochemical "pabulum" induces a dysfunction of the axonal membrane that can be modified by HD (Kiernan, 2002). Accordingly, nerve conduction studies across an HD session disclosed correlations between nerve excitability and serum potassium levels, thus demonstrating a strong contribution of hyperkalemia in the development of uremic neuropathies by inducing a chronic depolarization (Krishnan, 2006; 2006b, 2007).

Disregarding the pathogenetic link between uremia and peripheral neuropathy, the data discussed above show that this neurological abnormality is extremely common in ESKD and may be quickly reversed by kidney transplantation. Accordingly, given our result of a strong link between evidence of peripheral neuropathy and occurrence of RLS, this connection may be regarded as another intrinsic relation between RLS and renal failure, together with the further evidence of an amelioration of both RLS and peripheral neuropathy induced by kidney replacement.

Laboratory data

In the studies on uremic RLS, comparably to the evidences on idiopathic RLS in which low iron and ferritin levels are a well-known risk factors (Allen, 2003; AASM, 2005), great attention has been devoted to investigate the potential association with anemia and/or iron deficiency. Accordingly, the first study on uremic RLS found an association with anemia

(Roger, 1991), that was confirmed by some subsequent investigations (Bastos, 2006; Araujo, 2010; Sabry, 2010; Salman, 2011), but largely not replicated (table 3).

In our study, we did not found an association between RLS and anemia, but we highlight a non-significant trend for lower iron stores in ESKD patients with RLS as suggested by the lower levels of iron and ferritin. Indeed, we emphasize that in the clinical management of ESKD, all our patients were chronically supplemented with iron and erythropoietin, thus probably reducing the differences between patients' subgroups. In light of this consideration, we would have probably obtained more useful information from the global iron / erythropoietin load needed to maintain an adequate iron metabolism than from the final results of this balance documented by laboratory data. Previous authors showed that RLS symptoms severity was decreased by efficacious erythropoietin / iron replacement (Roger, 1991), that the occurrence of periodic leg movements during sleep significantly decreased with the same approach (Benz, 1999), and that the supplementation of iron e.v. in a double blind design proved a significant, albeit transient, amelioration of RLS symptoms severity (Sloand, 1994). Given the cross-sectional design of our data collection of treated ESKD patients, and the absence of information on the global load of iron replacement therapy, we cannot exclude that iron deficiency influenced the pathogenesis of RLS in our patients. However, in our cross-sectional observation, we highlight that the laboratory parameters of iron assessment (i.e. the final balance of iron metabolism) did not appear to play a major role for the occurrence of RLS in well-treated ESKD patients.

Other authors disclosed different associations between RLS and high levels of urea (Walker, 1995; Kim, 2008), high levels of creatinin (Walker, 1995), high (Mucsi, 2005) or low (Collado-Seidel, 1998) levels of parathyroid hormone, high levels of calcium (Rijsman, 2004; Kawauchi, 2006), and high levels of phosphorus or low levels of albumin (Sabri, 2010). Conversely, most studies did not replicate the above findings (table 3). In line with the above literature, we did not found significant associations with any of the above laboratory

parameters but only non-significant trends towards lower levels of albumin, and higher levels of potassium, thus excluding a prominent role for these biochemical variables on RLS pathophysiology.

Intriguingly, when analyzing separately the data collected in the S.Orsola Dialysis Unit, we disclosed an association between RLS and biochemical evidence of chronic inflammation (i.e. higher levels of erithrosedimentation rate, white cells count, and fibrinogen) (La Manna, 2010), that did not reached the statistical significance in the whole study sample. We consider however this partial, but novel, association of high interest, given the impact of inflammation on other biochemical indexes such as ferritin, as well as the complex interplay already proven between sleep disorders such as obstructive sleep apnea, insomnia or sleep restriction and inflammatory markers (Vgontzas, 2003; Mullington, 2010). In line with this association, some studies on ESKD patients showed elevated levels of proinflammatory cytokines in patients with sleep complaints with polysomnographic evidence of sleep-disordered breathing (Erten, 2005), in patients with subjective poor sleep quality undergoing peritoneal dialysis (Yang, 2007) or HD (Chiu, 2008), and in HD patients complaining of insomnia (Bornivelli, 2008). None of these studies however specifically assessed RLS as a potential cause of the sleep complaints within the reported association with biochemical evidence of inflammation. We consider our finding on the first 100 patients as a clinical caveat for considering also inflammation and its complex interplay with nocturnal sleep and with other biochemical parameters when evaluating factors influencing RLS occurrence. However, our results seem to exclude a prominent role for inflammation on RLS pathophysiology in ESKD patients.

Table 2: overview of the reported associations between RLS and clinical features in ESKD patients

	Female sex	**Age**	**Comorbidity**	**Peripheral Neuropathy**
Presence of Association	Roger 1991; Walker 1995; Siddiqui 2005; Al-Jahdali 2009; Araujo 2010.	Unruh 2004 (with higher age); Kawauchi 2006 (with lower age).	Unruh 2004 (diabetes); Mucsi 2005 (comorbidity count); Telarovic 2007 (diabetes); Al-Jahdali 2009 (diabetes); Araujo 2010 (CCI).	Cirignotta 2002 (signs are confounders).
Absence of Association	Huiqi 2000; Unruh 2004; Mucsi 2005; Merlino 2006; Bastos 2007; Kim 2008; Sabry 2010; Salman 2011.	Walker 1995; Winkelman 1996; Collado-Seidel 1998; Huiqi 2000; Mucsi 2005; Merlino 2006; Bastos 2007; Kim 2008; Al-Jahdali 2009; Araujo 2010; Salman 2011.	Unruh 2004 (comorbidity load); Kim 2008 (diabetes); Bastos 2007 (diabetes); Salman 2011 (additional diseases).	Roger 1991 (neurol.examination); Rijsman 2004 (EMG/neurol.exam.); Winkelman 1996 (single nerve EMG); Araujo 2010 (clinical records).

Table 3: overview of the reported associations between RLS and laboratory data in ESKD patients

	Iron / Anemia	**Laboratory data**
Presence of Association	Roger 1991 (Hb); Bastos 2006 (Iron); Araujo 2010 (Hb, not ferritin); Sabry 2010 (Hb); Salman 2011 (Hb).	Walker 1995 (↑Urea, Creatinin); Collado-Seidel 1998 (↓PTH); Rijsman 2004 (↑Calcium); Mucsi 2005 (↑PTH); Kawauchi 2006 (↑Calcium); Kim 2008 (↑preHD Urea); Sabri 2010 (↑phosphorus-↓albumin).
Absence of Association	Walker 1995; Winkelman 1996; Collado-Seidel 1998; Huiqi 2000; Takaki 2003; Unruh 2004; Rijsman 2004; Mucsi 2005; Siddiqui 2005; Kawauchi 2006; Bastos 2007; Hsu 2008; Kim 2008; Al-Jahdali 2009.	Winkelman 1996; Takaki 2003; Unruh 2004; Siddiqui 2005; Al-Jahdali 2009; Araujo 2010; Salman 2011.

Predictors of RLS occurrence in ESKD patients

After searching for univariate associations with RLS, we merged together all the collected evidences in order to refine a multivariate model able to predict RLS occurrence in our ESKD population. Our final results confirmed that positive first-degree family history, high risk of peripheral neuropathy, and reduced to absent residual renal function were the main predictors of RLS.

According to the above discussion of each specific association with RLS, our model suggests that RLS in the course of ESKD may be regarded as the result of a complex interaction between a pre-determined genetic predisposition (family history resuming in itself the role of female sex) and environmental factors intrinsically linked to the kidney purification function (residual renal function) or secondary to ESKD (peripheral neuropathy).

Moreover, when analyzing the different subgroups of RLS patients, we also disclosed several evidences further supporting our global findings. First, one third of patients recalled RLS starting before any evidence of kidney disease, being probably more "idiopathic" than "uremic" in nature. Indeed, RLS patients with positive family history also started earlier to complain RLS symptoms, either before a kidney diagnosis, or during a milder stage of kidney insufficiency not requiring HD. Second, the patients with RLS arising before the interview, but already disappeared at the time of the study, were less afflicted by the two kidney related factors (peripheral neuropathy and residual renal function), strongly confirming their role for RLS occurrence. Conversely, patients with RLS arising after HD were less commonly having a positive family history and reported its onset later.

In our perspective, we may speculate that RLS in ESKD is a complex mixture of patients with different levels of genetic background on which several environmental factors can play a pivotal role in overcoming an hypothetical "threshold" differentiating subjects with and without active RLS symptoms. Other clinical conditions such as iron deficiency (Allen, 2007) or pregnancy (Manconi, 2004) could be regarded as similar functional and reversible clinical

conditions all affecting RLS occurrence. Similarly, we may speculate that the concept of "transient" RLS during pregnancy as a risk factor for later in life stable RLS may corroborate the idea of a "threshold" for RLS symptoms occurrence that may be modulated by specific factors in the short- to mid-term, as well as by age in the long-term, comparably to the idiopathic form (Cesnik, 2010).

Impact of RLS on sleep comorbidity and daytime symptoms (sleepiness and fatigue)

In our study we systematically assessed symptoms of other sleep disorders using a semi-structured clinical interview or self-administered validated questionnaires. In particular, we evaluated the presence of insomnia, nocturnal leg cramps, sleep related eating disorder, nocturnal smoking, sleep disordered breathing as commonly occurring sleep-related disorders, and of daytime sleepiness and of daytime fatigue (together with individual chronotypology) as daytime consequences (and modulating factors).

Concerning nocturnal complaints, our results excluded an overlap of RLS with sleep disordered breathing and nocturnal leg cramps, but showed a strong association with insomnia. The comorbidity of RLS with insomnia is well-grounded in the idiopathic form, where current knowledge considers RLS as the cause of sleep onset and sleep maintenance insomnia up to the definition of RLS sufferers as "nightwalkers" (AASM, 2005). Our results is perfectly in line with previous studies in ESKD patients, in which RLS has been consistently associated with insomnia (Mucsi, 2005; Merlino, 2006; Al-Jahdali, 2009; Araujo, 2010; Sabri, 2010), and with subjective poor sleep quality (Walker, 1995; Winkelman, 1996; Kawauchi, 2006; Unruh, 2006; Bastos, 2007; Araujo, 2010), in the absence of studies not confirming a detrimental impact of RLS on sleep initiation and continuity.

For the first time, we assessed the potential association of RLS with sleep related eating or smoking, given the previously reported association in the idiopathic form (Provini, 2009; 2010). Our results showed a clear tendency for these two nocturnal compulsive sleep related

behaviours to occur more frequently in patients with RLS, but however without reaching the statistical significance, thus differing from what previously found in idiopathic RLS.

Concerning daytime symptoms, we did not found a significantly increased prevalence of excessive daytime sleepiness according to the Epworth sleepiness scale (Johns, 1991), but in our patients with RLS the severity of the syndrome correlated to the degree of daytime sleepiness, thus suggesting a detrimental role in RLS sufferers. Previous studies reported conflicting results on this topic, with several authors disclosing a significant association (Walker, 1995; Winkelman, 1996; Merlino, 2006; Al-Jahdali, 2009; Araujo, 2010), and other works not confirming the impact of RLS on sleepiness (Bastos, 2007; Kim, 2008). Therefore, we believe that daytime sleepiness is a common and complex complaint in ESKD (Parker, 2003), but may be ascribed to factors other than RLS; however, when RLS is present, it may further contribute to worsen daytime functioning. We also must keep in mind that the subjective sleepiness evaluation with the Epworth sleepiness scale has not been yet validated in the context of chronic medical illnesses with high levels of comorbidities such as ESKD, and in the context of ESKD the subjective score was found to be unrelated to objective sleepiness measures (Parker, 2003).

Many ESKD patients undergoing HD also complain of daytime fatigue, a subjective sense of weakness, lack of energy and tiredness. Fatigue semantically overlaps with daytime sleepiness and depression also sharing with EDS the difficulty of being adequately recognized with validated tools in this patient population (Jhamb, 2008). To our knowledge, studies evaluating fatigue in light of coexistent sleep disturbances in ESKD are lacking, and we found non-significantly increased fatigue levels in our RLS patients. Similarly to daytime sleepiness, we disclosed a significant correlation between RLS severity and fatigue that still indicated a detrimental role for RLS. Given the potential interplay between fatigue and sleepiness, as well as the potential semantic overlap, we emphasize that in our population there was no

correlation between the two symptoms' scores (r=0.099, p=0.314, data not shown), that could be therefore regarded as different aspects of daytime functioning.

Impact of RLS on mood and quality of life

In our population, we disclosed a significant association of RLS with lower quality of life and higher levels of depression. More in detail, patients with RLS had lower scores in all the domains of the SF-36, but the mental health one. Additionally, all the scores of the different SF-36 areas pertaining to the physical and mental components (but the mental health) were negatively correlated with RLS severity in the patients with RLS. Similarly, the depression scores were positively correlated with RLS severity in RLS patients. Therefore, RLS negatively impacted on the physical and mental perception of the quality of life as well as on mood of our ESKD patients, and its severity seemed to modulate these aspects in a dose-dependent fashion.

A few of the studies on uremic RLS have assessed the impact of the syndrome on different aspects of mood and quality of life showing mostly a negative impact, despite the different methods used to make the diagnosis of RLS (in terms of criteria applied, as outlined in table 1) and to measure the outcomes (various questionnaires). Based on these differences, it is somehow difficult to compare in detail the results of the literature with our findings.

Concerning the assessment of the quality of life, all authors reported a negative impact of RLS, but with different levels of significance pertaining the specific domains. Unruh et al, evaluating by means of a single question (questionnaire) 894 HD patients, reported a negative impact on both the physical and mental composite scores of the SF-36 (Unruh, 2004), whereas Kawauchi et al, evaluating a cohort of 228 HD patients applying outdated RLS criteria (Walters, 1995) by means of a questionnaire assessment, disclosed a negative impact on the BP, GH, VT, RE, MH domains, leading to a global impairment of the mental

composite score, but not of the physical composite score of the SF-36 (Kawauchi, 2006). In between the two previous reports, Mucsi et al evaluated a cohort of 333 HD patients by means of a questionnaire applying the four RLS criteria, and reported a negative impact of RLS on all the domains of the SF-36, but not on the PF and VT ones (Mucsi, 2005). Finally, a negative impact of RLS on all the SF-36 domains was also disclosed in a population of 785 kidney transplanted patients by means of a questionnaire assessment (Molnar, 2007b).

Two polysomnographic studies evaluated the relation between sleep complaints, objective findings at the nocturnal recording, and quality of life. Parker et al assessed 46 HD patients carefully selected to exclude subjects with sleep complaints and disclosed negative correlations between the index of PLMS and different dimensions of a quality of life index (Parker, 2003). Conversely, Rijsman et al recorded 48 ESKD patients undergoing HD or peritoneal dialysis and disclosed a prevalence of 58% of RLS, of 85% of periodic limb movement disorder (index of PLMS>5) or of 71% of clinically significant periodic limb movement disorder (index of PLMS>25). Patients with RLS had more PLMS, and the association between PLMS and RLS leaded to higher level of depression at the "sickness impact profile", an instrument measuring quality of life (Rijsman, 2004).

Few studies aimed at evaluating the relation between RLS and mood reporting discordant results. Takaki et al evaluated 490 HD patients by means of a questionnaire assessment applying the 4 RLS criteria (Walters, 1995) together with the "hospital anxiety and depression scale", and did not found any association between RLS and depression, but an impact of RLS on anxiety and coping/avoidance strategies (Takaki, 2003). Conversely, in a recent study on a mixed population of 788 transplanted and 161 HD patients evaluated with a questionnaire applying outdated RLS criteria (Walters, 1995), the authors reported that RLS affected 5% and 11% of transplanted and waitlisted patients respectively, with a negative impact on depression and insomnia assessed by means of the "center for epidemiologic studies - depression scale" and of the "Athens insomnia scale" (Szenkiralyi, 2009). Finally, Araujo et

al evaluated by means of a questionnaire assessment using the latest RLS criteria (Allen, 2003) a population of 400 HD patients, and disclosed higher levels of depression at the first version of Beck depression inventory translated in Portuguese in RLS vs non-RL sufferers (13.0 vs 9.6, $p<0.000$) (Araujo, 2010).

According to the above cited literature, none of the authors evaluated simultaneously quality of life and depression in relation to RLS in ESKD patients. Conversely, some authors explored the relation between sleep quality without further investigating the underlying sleep complaints and these psychological outcomes. Iliescu et al studied 81 HD patients with the Pittsburg sleep quality inventory, a measure of insomnia and sleep quality, disclosing negative correlations with the mental and physical composite scores of the SF-36. Moreover, the authors reported that subjects with "bad" sleep at the questionnaire were more frequently "depressed" (i.e. used antidepressant therapies) and had lower SF-36 scores (Iliescu, 2004). Similarly, other authors reported a progressive decrease of sleep quality during the first year of HD that was correlated with both the physical and mental composite scores of the SF-36, and with the additional evidence of a negative impact of this sleep quality decline on survival (Unruh, 2006). Similarly, other authors evaluating the complaint of insomnia found a negative impact of this sleep complaint on mood (Williams, 2002; Bornivelli, 2008; Paparrigopoulos, 2009), as well as on the physical and mental composite scores of the SF-36 (Paparrigopoulos, 2009).

Impact of RLS on the occurrence of new cardiovascular events and survival

In the general population RLS has been associated with increased prevalence of cardiovascular diseases (Ulfberg, 2001; Ohayon, 2002; Winkelman, 2006; 2008), and the unanswered question whether RLS may impact on mortality is one of the actual hottest topics in sleep research (Schlesinger, 2009; Walters, 2009).

In the clinical setting of ESKD, previous reports disclosed a detrimental impact of RLS on mid-term prognosis, with lower survival rates at 2.5 years (Winkelman, 1996), and at 4 years of median follow-up (Unruh, 2004). Moreover, RLS has been associated with higher mortality at 4-year follow up also in kidney transplanted patients (Molnar, 2007). These studies, however, did not provide further insights on the cause of death, as long as this factor was not assessed (Winkelman, 1996), or the cardiovascular cause of death did not appeared to be associated with RLS (Unruh, 2004). Nevertheless, Winkelmann et al disclosed an association between RLS and "signs off" (i.e. precocious HD discontinuation), thus supporting the notion that RLS may worsen survival being a factor that contributes to inefficacious HD procedure (Winkelman, 1996), a finding that has been replicated in patients undergoing peritoneal dialysis (Hui, 2000), as well as in our cohort.

In our study (La Manna, 2010), we confirmed the independent impact of RLS on survival at a shorter follow up (18 months), adjusting for the other common risk factors such as age, sex, comorbidity, and biochemical parameters associated with lower survival including absent residual diuresis. Moreover, we also showed that the highest risk was associated to RLS with "continuous" vs "intermittent" course, a finding that sounds in line with studies showing an increasing association of cardiovascular diseases to RLS patients with more frequent (up to daily) occurrence of symptoms (Winkelman, 2006; 2008).

The second main finding of our longitudinal branch of the study is the association between RLS at baseline and occurrence of new cardiovascular events at 18 months follow-up (La Manna, 2010). Regarding the further dissection of RLS in subgroups with "intermittent" vs. "continuous" course, we disclosed that the latter groups had an higher (more than two-fold) occurrence of new cardiovascular events whereas the former had an 80% increase when compared to ESKD patients without RLS, but these findings did not reach the statistical significance. The evidence of increased occurrence of new cardiovascular events is of great interest from a mechanistic perspective, trying to establish a link between RLS and lower

survival. However, despite proving an impact of RLS on both mortality and occurrence of cardiovascular events, we were not able to prove that RLS was significantly associated to a cardiovascular cause of death in our population (19% vs 17%, $p>0.05$, data not shown).

The association between chronic renal failure and cardiovascular comorbidities has been extensively documented in the scientific literature (Parfrey, 1999; Shastri, 2010; Foley, 2007; Van der Zee, 2009). Cardiovascular disease is the well-established leading cause of morbidity and mortality in patients with chronic kidney disease with an increasing burden related in a dose-dependent fashion to worsening renal function (Shastri, 2010). Within the clinical spectrum of chronic kidney failure, the traditional risk factors such as age, diabetes, hypertension or dyslipidemia play an important role in patients with mild to moderate renal disease. Conversely, in the patients with ESKD requiring HD, non-traditional and novel risk factors including inflammation, oxidative stress, vascular calcification appear to confer additional burden and deserve careful consideration (Van der Zee, 2009). In this context, RLS may be regarded as a new and potentially treatable risk factor.

To date, the scientific literature provides well-grounded evidences on the connections among RLS, periodic leg movements during sleep (PLMS), transient rises of heart rate (Sforza, 1999; Ferri, 2007; Guggisberg, 2007), and arterial blood pressure (Alì, 2001; Pennestri, 2007; Siddiqui, 2007), probably mediated by sympathetic overactivity and also leading to measurable changes of cerebral hemodynamics proved by transcranial Doppler (Droste, 1996), and cerebral near infrared spectroscopy (Pizza, 2009). Based on this, and on the strong detrimental effect of nocturnal hypertension on the cardiovascular system, some authors recently hypothesized that nocturnal hypertension may contribute to increased cardiovascular risk in dialysis patients with RLS (Portaluppi, 2009). RLS may directly cause nocturnal hypertension by PLMS with subsequent development of daytime hypertension as proved for obstructive sleep apnea syndrome (Wolf, 2010). To our knowledge, however, the presence of a non-dipping status has been proven only in a single study of children with PLMS (Wing,

2010). In patients suffering from daytime hypertension, an association with higher prevalence of PLMS was reported (Espinar-Serra, 1997), suggesting a "paradoxical" top-down role for hypertension itself in the development of RLS/PLMS. Given the lack of association between RLS and hypertension in some studies (Winkelman, 2006; 2008), other mechanisms such as increased atherosclerotic plaque formation and rupture or confounding comorbidities have been postulated to link RLS to CVD (Walters, 2009). In uraemic patients with low plasma levels of tyrosine (amino acid precursor to dopamine) (Fürst, 1989), we may speculate that overall reduced dopaminergic activity could underlie the pathogenesis of both RLS and hypertension through different levels of the nervous and renal systems (Hussain, 2003).

In the field of ESKD, Benz et al performed a polysomnographic study of patients reporting disrupted nocturnal sleep or daytime sleepiness and provided additional evidences connecting the index of PLMS to lower survival (Benz, 2000). RLS patients quite constantly presents with PLMS (AASM, 2005), and patients with uremic RLS seem to have a more severe and sleep disrupting form of the disease when polygraphically recorded, especially in terms of PLMS frequency and overnight distribution (Wetter, 1998; Enomoto, 2008). Moreover, polysomnographic data of ESKD patients carefully selected for the absence of subjective sleep complaints disclosed the frequent occurrence of PLMS (Parker, 2003b), whereas patients with subjective report of RLS associated with nocturnal evidence of PLMS had the poorest objective sleep quality measures (Rijsman, 2004).

To establish the directional links between RLS, PLMS, cardiovascular diseases and survival, further longitudinal studies are warranted, with the need to objectively document nocturnal sleep and autonomic nervous system alterations together with detailed clinical information on sleep complaints. Finally, a double-blind trial approach could also be necessary to test whether RLS plays a primary and, therefore, reversible role in this context, or whether RLS should be regarded as an epiphenomenon that tell-tales a more severe general health condition.

Chapter 5: Conclusion

The current research project aimed at assessing the prevalence and impact of RLS in a large cohort of ESKD patients using validated and reliable tools in order to avoid the confounding effect of RLS "mimics". Indeed, we found a prevalence rate of over 30%, thus far higher than the data reported in the general population, and not biased by other potential clinical conditions.

For the first time, we performed a systematic and comprehensive assessment of clinical, biochemical, kidney-related factors, and sleep-comorbidity in order to verify which features of ESKD may be related to the occurrence of RLS. Our results disclosed a complex context in which the genetic background for RLS (in terms of positive first degree family history), the neurological evidence of peripheral neuropathy, and the most severe condition of renal function (in terms of residual renal function) contribute all together to the occurrence of RLS. Therefore, the pathophysiology of RLS in the course of ESKD involves a complex interplay of "idiopathic" (i.e. genetic background) and "secondary" factors, the latter being intrinsic to the metabolic condition (i.e. residual renal function) as well as secondary to kidney insufficiency (i.e. polyneuropathy). Indeed, clinical history of RLS in our patients also disclosed the arise of the disorder in different periods, ranging from before any evidence of kidney disease, to the phase of chronic kidney failure and after the initiation of hemodialysis. Moreover, our finding of an association with residual renal function and peripheral neuropathy may well explain the amelioration or resolution of RLS after successful kidney transplantation in parallel with the quickly restored purification function provided by the graft and with the amelioration of the peripheral neuropathic condition.

Finally, we confirmed the detrimental impact of RLS on different aspect of patients well-being. In particular, RLS disrupts nocturnal sleep for the clinical complaint of insomnia, as well as negatively influences quality of life and mood. Despite the absence of an association

with daytime sleepiness and fatigue, RLS may contribute to worsen these aspects of daytime functioning at least in RLS sufferers. From a medical care standpoint, a notable finding of the current research project has been the demonstration of a detrimental effect of RLS on the occurrence of new cardiovascular events and survival, despite comparable levels of medical comorbidities at baseline evaluation. The latter observation may be regarded as a step forward in the mechanistic comprehension of the relation between RLS and medical outcomes, and warrants further longitudinal studies assessing the effect of RLS treatment.

To conclude, RLS is a complex disorder. The condition of RLS in the course of ESKD disclosed several factors that should be carefully evaluated in future studies on "idiopathic" RLS. Conversely, in the clinical management of ESKD a careful evaluation of RLS should be routinely performed in light of its potentially treatable impact on clinical outcome.

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