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Severe hepatocytotoxicity linked to denosumab

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Abstract. – OBJECTIVE: Denosumab (Prolia, Amgen, Thousand Oaks, CA, USA) is a fully human antibody to the receptor activator of nuclear factor-KB ligand (RANKL). We present a case of submassive hepatic necrosis with evidence implicating cytokine induction resulting from an immune reaction to denosumab.

CASE REPORT: A 72-year-old lady presented with elevated liver enzymes. One month previously, she received a s/c administration of 60 mg of denosumab. Viral hepatitis A, B and C and human herpes viruses 6-7 were negative as were routine autoimmune serology. Transaminases reached more than 50 x ULN, and gamma-glutamyl-transpeptidase (GGT) increased to more than 30 x ULN. Serum bilirubin reached 13.8 mg/dL. The serum albumin level decreased to 2.8 g/L. Prednisone (40 mg) and ursodeoxycholic acid (900 mg) were administered. The Naranjo Adverse Drug Reaction probability score was 6, consistent with a probable adverse drug reaction. A liver biopsy revealed sub-massive hepatic necrosis consistent with drug-induced liver injury (DILI). During steroid tapering, there was a slow decline in the levels of both the transaminases and the GGT, and a concomitant increase in the serum albumin. A month after stopping prednisone and ursodeoxycholic acid, there was an acute increase in the level of the transaminases and a decrease in the serum albumin. Steroid reintroduction resulted in normalization of the liver enzymes and synthetic capacity. A lymphocyte toxicity assay to denosumab was demonstrated a hypersensitivity reaction to denosumab resulting in 31% toxicity. The control patient showed no toxicity to denosumab. Cytokine levels (pg/mL) were as follows: Interleukin (IL)1 was 1193 (normal-24.5), IL8 357 (20-60), RANKL 224 (60-80), RANTES 215 (15-50), TNF-α 850 (25-50), TGF-β 546 (20-40), VEGF 735 (25-30). Serum RANKL was markedly reduced in the presence of denosumab (16 pg/mL). The elevated markers of apoptosis ccK18(M-30)(68-132) 140 IU and K18 apoptosis+ necrosis (M65) (62-213) 322 U/L implicate necrosis.

CONCLUSIONS: We suggest that RANKL inhibition can produce severe hepatic necrosis together with an increase in proinflammatory cytokines.

Key Words:
Apoptosis, Cytokine, Chemokine, Denosumab, Drug-induced hepatotoxicity, Necrosis, Nuclear factor-kB (NFkB), Osteoporosis, Receptor activator of nuclear factor-kB ligand (RANKL).

Abbreviations

ADR = adverse drug reaction; ALT = alanine aminotransferase (serum glutamic-pyruvic transaminase, SGPT); ALP = alkaline phosphatase; AST = aspartate aminotransferase (serum glutamic-oxaloacetate aminotransferase, SGOT); CB = conjugated (direct) bilirubin; CXCL8 = IL-8, Monocyte-derived Neutrophil Chemoattractant Factor, Interleukin 8; CYP = cytochrome P450; DILI = drug-induced liver injury; ELISA = enzyme-linked immunosorbent assay; GGTg = glutamyl-transferase (g-glutamyl transpeptidase, GGTP); HSR = hypersensitivity syndrome reaction; IFN-g = Interferon Gamma, Immune interferon; IL = interleukin; INR = International Normalized Ratio; LTA = lymphocyte toxicity assay; MTT-3-(4,5-dimethylthiazol-2-yl)-2,5-diophenyl tetrazolium bromide; NFKB = nuclear factor kappa-B; NKT, natural killer cells; PDGF = Platelet-Derived Growth Factor; RANK = receptor activator of nuclear factor kappa B ligand (RANKL); RANK-L = ligand of RANKRVE-representative volume element; RANTES = regulated upon activation, normal T-cell expressed and secreted; TB = Total Bilirubin (summation of conjugated and non-conjugated serum bilirubin); TGF-β = transforming growth factor beta; TNF-α = tumor necrosis factor alpha; ULN = Upper Limit of the Normal reference range; VBDS = vanishing bile duct syndrome.

Introduction

Postmenopausal osteoporosis results from bone loss and decreased bone strength mediated by an

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increased rate of bone remodeling secondary to reduced estrogen levels[1-4]. Remodeling cycles are initiated by osteoclasts. Cytokines are intracellular messengers that regulate the immune system. Cytokines inhibitors have been developed as novel therapies. Denosumab is a fully human antibody to the receptor activator of nuclear factor-kB (NFkB) ligand (RANKL). The therapeutic effect of denosumab rests on its capability to inhibit osteoclast differentiation. RANKL blocks the formation, activation, and survival of osteoclasts[5]. Prolia is a well-tolerated medication and there are no previous reports of hepatotoxicity. In addition, the RANKL pathway has no recognized effect on the liver. We report here a case of sub-massive hepatic necrosis most likely caused by denosumab. Hepatotoxicity with use of denosumab may be rare, but it is a clinically significant phenomenon.

Case Report

A 72-year-old lady was referred for investigation of elevated liver enzymes. She suffered from osteoarthritis of the left hip and was due to undergo a hip replacement. Her medication consisted of occasional non-steroidal anti-inflammatory drugs (NSAIDS), amlodipine and bisoprolol for hypertension. One month before referral she had received a subcutaneous administration of 60 mg of denosumab for osteoporosis. Physical examination was unremarkable. Laboratory investigations revealed a complete blood count (CBC) and international normalized ratio (INR) within normal limit and an elevation of serum transaminases to around 5 times the upper limit of normal (ULN). Routine blood tests for determination of hepatitis, B and C were negative as were routine autoimmune serology. IgG seropositivity was detected for hepatitis A virus infection. Serum ceruloplasmin, ferritin and alpha-1-antitrypsin levels were within normal limits. Thus there was no evidence of a previous chronic liver disease. Additionally we excluded the possible reactivation of human herpes viruses 6 and 7 (HHV-6, HHV7), previously described in hypersensitivity syndromes to drugs. During 6 weeks of follow-up there was a slow increase in her serum transaminases to 12 times the ULN and then subsequently an elevation to 1500 IU/mL (Figure 1). The gamma-glutamyl transferase (GGT) level was elevated to 755 U/L. She became icteric with a bilirubin of 10 mg/dL. Her INR was elevated to 1.79 and her serum bilirubin increased to 13.8 mg/dL. The INR was not corrected by administration of vitamin K intravenously and her serum albumin level decreased to 2.8 g/L. At this stage, a liver biopsy was performed. The liver biopsy revealed inflammation of hepatic tissue, sub-massive hepatic necrosis and bile duct proliferation consistent with drug-induced liver injury (DILI) (Figures 2A and 2B). The reticulin staining enhanced the presentation of the necrotic tissue (Figure 3). Moreover, immunohistochemistry staining with anti-cytokeratin antibody, clearly showed necrotic clusters of cells (Figure 4). Oral prednisone 40 mg once daily was administered. During the next 3 weeks the INR decreased to 1.3 and the albumin initially rose to 3 g/dL, but then declined to 2.7 the bilirubin level decreased to 7.8 mg/dL. Ursodeoxycholic acid 300 mg two times per day was added. Over the course of the following months there was a slow decline in the levels of both the transaminases and the GGT and a concomitant increase in the serum albumin. There followed a slow tapering of the steroids and also the ursolit was stopped. A month later there was a large increase in the level of the transaminases and a decrease in the serum albumin from 3.2 to 2.9 mg/dL. The prednisone was reintroduced at a dose of 25 mg per day and the ursolit at 600 mg per day. Subsequently, there

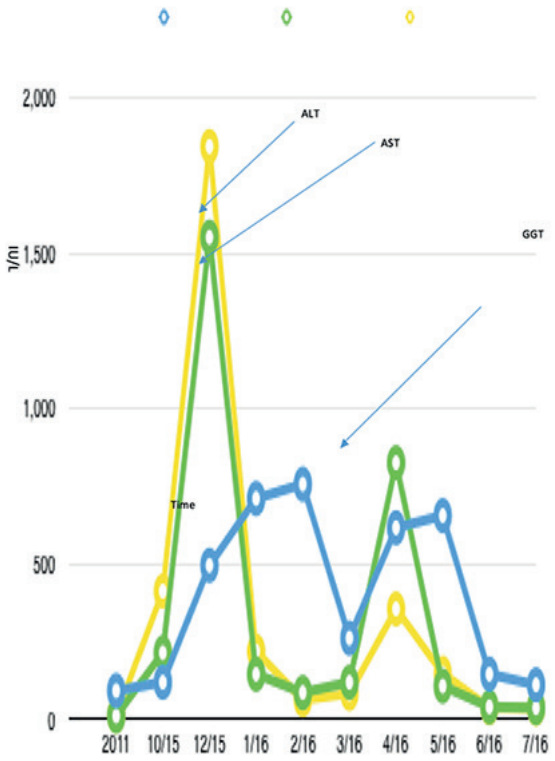

Figure 1.

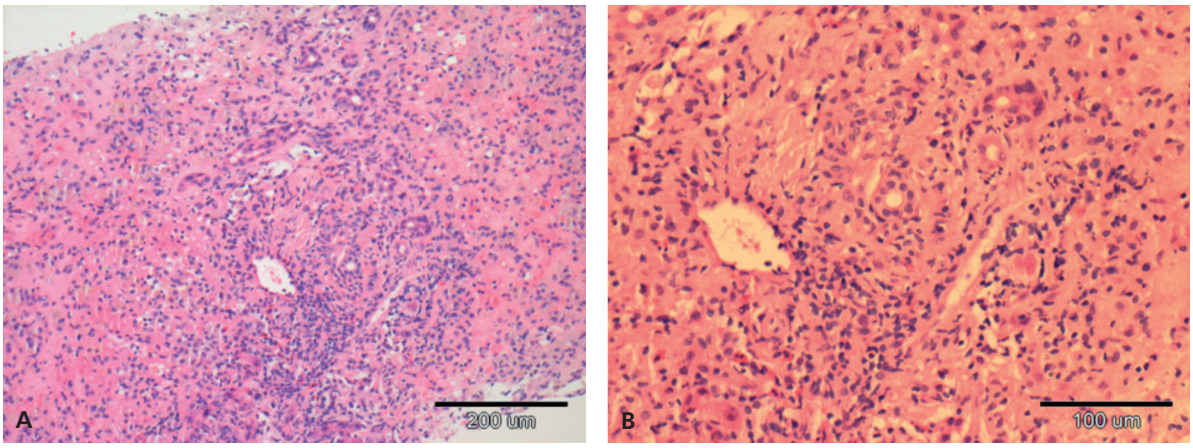

Figure 2. ***A,*** Hematoxylin and eosin stain (X75) showing massive liver cell necrosis associated with eosinophil infiltration and bile duct proliferation. ***B,*** Liver cell necrosis associated with acute inflammation with eosinophylia in the portal tracts. Bile duct proliferation and signs of cholangitis.

was a decrease in the transaminases again over a 3-month-period with slow tapering off of the steroids. Currently, she remains in good health on a maintenance therapy of prednisone 5 mg per day and ursodeoxycholic acid 600 mg per day (15 mg/kg). To identify a correlation between medications received and possible liver injury, we examined the temporal relationship to the beginning of the biologic therapy, the amount administered, and the duration of use. This also included over-the-counter medications, as well as herbal and dietary supplements. Lack of illness before ingesting denosumab, clinical illness and biochemical abnormalities developing after beginning the biologic, as well as an improvement after the drug was withdrawn incriminates denosumab in DILI. Since the illness may recur upon reintroduction of the offending substance rechallenge is not ethical. To determine if the liver induced injury was related to the biologic therapy we performed a lymphocyte toxicity assay (LTA) to denosumab. We aimed to identify cytokines as biomarkers for the prognosis and diagnosis of DILI caused by a new biological drug. Since denosumab is an inhibitor of RANKL and, therefore, can activate an immune inflammatory response, we measured the levels of cytokines and chemokines in the sera of the patient.

Methods

Lymphocytes were extracted following the centrifugation of fresh patient's blood, using a density gradient. Patient's lymphocytes were incubated with therapeutic concentrations of

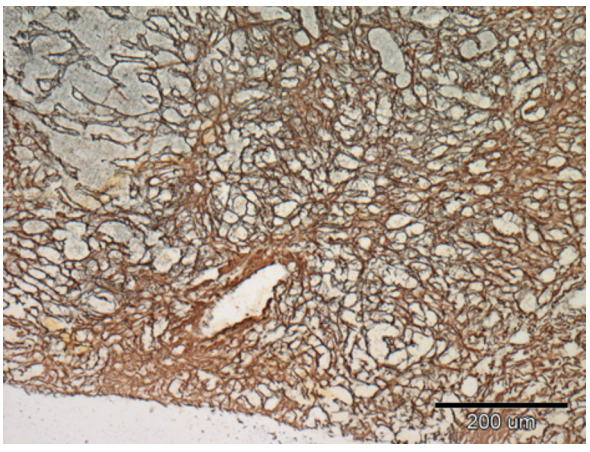

Figure 3. Reticulin stain (x75) showing massive liver cell necrosis.

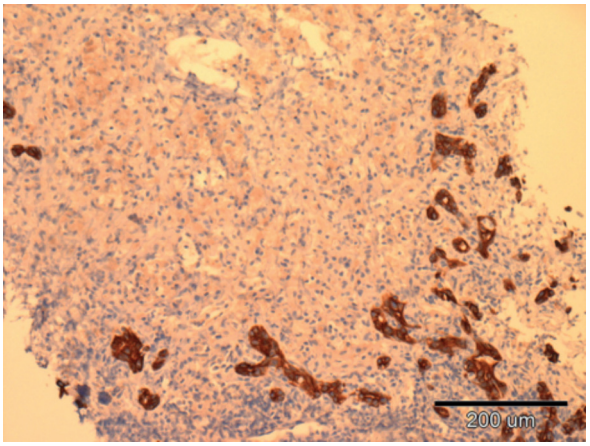

Figure 4. Imunohistochemistry: cytokeratine-17 (CK-17) highlighting the bile ductular proliferation.

the denosumab. Following 24 hours incubation, the cell culture supernatant was removed, and lymphocytes were further incubated with the yellow tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The degree of MTT reduction to the water-insoluble purple formazan represents a measure of succinate dehydrogenase activity, which in turn indicates cellular viability[6]. Toxicity to the biologic therapy was expressed as the percentage of reduction in dye intensity relative to the control (patient's cells and metabolizing system in the absence of drug) and corrected for toxicity due to drug alone (in the absence of metabolizing system). Moreover, in parallel with this sample, we performed the same determination on a sample from a control patient that did not have a reaction to denosumab. A lymphocyte toxicity assay will perform similarly during both a DILI episode and several years later, due to memory cells. The innate immune system also participates. Hepatocyte necrosis activates the innate system, most likely through proinflammatory mediators (e.g., cytokines, chemokines). These mediators can be directly cytotoxic or lead to recruitment of cells of the innate immune system. The innate immune system may also have a role in recovery via anti-inflammatory components. Cytokines play an essential role in the inflamed liver[7]. To analyze the mechanism of liver damage we examined a panel of cytokines. TNF-a levels (pg/mL) were quantitatively determined in serum, using enzyme-linked immunosorbent-assay (ELISA). Cytokine and chemokine levels were measured by ELISA as follows: TGF-b (R&D Systems, Inc.; Minneapolis, MN, USA), IL-1, VEGF, sRANKL (PeproTech Asia, Rehovot, IL, USA), IL-6, IL-8, RANTES, TGF-a (eBioScience, Frederick, MI, USA) with 96% sensitivity and 92% specificity. NF-kBp65 activity was measured by ELISA (Invitrogen Corporation, Carlsbad, CA, USA). The test was performed according to manufacturer specifications. RANTES plays a primary role in the inflammatory immune response via its ability to chemo-attract leukocytes and modulate their function. Aliquots from the sample were added to the 96-well plate. Each specimen was analyzed in duplicate with 95% sensitivity and 92% specificity. Our measurement system demonstrates strong correlations across replicates with correlation coefficients > 0.99, ensuring reliable detection of differences in cytokine levels between biological samples.

Apoptosis

Liver cell apoptosis is triggered by host conditions, including starvation, stressful environmental conditions and the presence of toxins[8]. To confirm the mechanism we choose to analyze multiple apopto-necrotic markers. Cytokeratin 18 encodes the type I, intermediate filament chain keratin 18. Keratin 18, together with its filament partner keratin 8, are perhaps the most commonly found members of the intermediate filament gene family. They are expressed in single layer epithelial tissues of the body. Mutations in this gene have been linked to cryptogenic cirrhosis. Two transcript variants encoding the same protein have been found for this gene[8]. We measured the cytokeratins in sera and tissue media using M30 and M65. M30 is specific for apoptosis and M65 combine death processes from both apoptosis and necrosis. In epithelial cells, one of those substrates is the intermediate filament protein keratin 18 (K18). The M30 antibody recognizes a neo-epitope exposed after caspase cleavage of K18 after the aspartic acid residue 396. Cleavage at this position occurs early during apoptosis by caspase 9 and during the execution phase by caspase 3 and caspase 7. The M30 Apoptosense® ELISA measures the levels of soluble caspase-cleaved K18 (ccK18) fragments containing the K18Asp396 neo-epitope. The ccK18 level increases during apoptosis and is inhibited by the caspase-inhibitor zVAD-fmk. M65 ELISA measures total K18. Combining the two assays M30 and M65 is useful for assessment of cell death by both apoptosis and necrosis. The markers cleaved cytokeratins CK 18 and CK 8 (M30 and M65) were quantified using kits from Bender MedSystems (Vienna, Austria). We used the same procedures described by us in previous studies[9]. The correlation coefficient was linear (r = 0.990). NFkB is predominantly localized in the cytoplasm with a double role as a mediator of: cell survival as well as up-regulator of Fas-FasL apoptosis. These markers had AUROC of 0.90 with 80% sensitivity and 90% specificity for detecting advanced fibrosis. We used standards and reference reagents available from the National Institute for Biological Standards and Controls (NIBSC, Herts, UK). These methods are standardized in our laboratory according to the procedures described[9]. The RealStar® HHV-6 PCR Kit 1.0 is an *in vitro* diagnostic test, based on Real-time PCR technology, for the detection, differentiation, and quantification of Human Herpes virus 6A (HHV-6A) and Human Herpes virus

6B (HHV-6B) as well as HHV-7 specific DNA. We used RealStar® HHV-6 PCR Kit 1.0, Altona Diagnostic GmbH (Hamburg, Germany) and Real-time PCR instrument Rotor-Gene™ 3000/6000 (Corbett Research) for our determinations[10].

Results

The LTA of the patient indicated 31% toxicity upon incubation with the monoclonal antibody *vs.* control. As control we examined the response of patient cells incubated under identical conditions in media not containing denosumab. Also, there was an elevation in the serum levels of several critical cytokines (Table I). The very high levels of both cytokines and chemokines in the patient's sera are consistent with the profound inflammation present in the liver. Moreover, the elevated level of transforming growth factor beta (TGF) may mediate the fibrogenic effect of the drug on the liver. Furthermore, levels of 2 markers of apoptosis were examined and found to be elevated – ccK18 (M-30) 140 IU/L (normal 68-132) and K18 apoptosis+ necrosis (M65) 322 IU/L (normal 62-213). This result is consistent with necrosis. Examining the levels of cytokines, chemokines, and apoptotic-necrotic markers in the sera of this patient reflect the status of the individual 3-4 months after the drug-induced liver injury, at the time of the blood sampling. This demonstrates prolonged immune stimulation consistent with the long half-life of denosumab. To examine the cytokine network at the time when the denosumab produced the active injury to the individual, we measured also the same parameters in the media in which the lymphocytes were exposed to denosumab, and this revealed a 31% toxicity to the drug. As shown in Table II, exposure of the patient's lymphocytes to denosumab severely reduced RANKL production concomitantly with an activation of other proinflammatory mediators and, therefore, an imbalance between the pro and anti-inflammatory cytokines leading to necrosis.

Table I. Serum levels of cytokines, chemokines, apoptosis and necrosis markers in the patient and control.

Cytokines	Normal values	Control	Patient
IL-1 pg/mL	24-50	26	1193
IL-6 pg/mL	30-60	32	36
IL-8 pg/mL	20-60	38	357
NFκB pg/m L	15-40	32	313
RANTES pg/mL	15-50	35	215
TNF-α pg/mL	25-50	45	850
TGF-β ng/mL	20-40	26	546
VEGF pg/mL	25-60	42	735
sRANKL pg/mL	60-80	90	224
ccK18 (M-30) U/L	68-132	56	140
ccK8 (M65) U/L	62-213	28	322

Table II. Levels of cytokines, chemokines, apoptosis and necrosis markers in the media in which the lymphocytes of the patient and control have been exposed to denosumab.

Cytokines	Normal values	Control	Patient
IL-1 pg/mL	24-50	22	983
IL-6 pg/mL	30-60	18	30
IL-8 pg/mL	20-60	28	517
NFκB pg/mL	15-40	22	416
RANTES pg/mL	15-50	15	215
TNF-α pg/mL	25-50	22	590
TGF-β ng/mL	20-40	12	56
VEGF pg/mL	25-60	22	135
sRANKL pg/mL	60-80	60	24
ccK18 (M-30) U/L	68-132	16	40
ccK8 (M65) U/L	62-213	18	318

Discussion

Denosumab has been approved for the treatment of postmenopausal osteoporosis (Amgen Inc. Thousand Oaks, CA, USA). Studies of postmenopausal osteoporosis show that denosumab suppresses osteoclast differentiation, thus restraining bone reabsorption[11]. Simulation studies[12] suggest that denosumab generates a short-term bone volume gain, followed by constant or decreasing bone volume. This evolution is accompanied by a decrease of the bone turnover rate and by secondary mineralization, leading to higher mineral concentration per bone volume and its receptor[13]. Preclinical studies[14] have defined the role of RANKL in bone remodeling and provided evidence for the therapeutic potential of RANKL inhibition in conditions of bone loss. We believe that this case is a unique example of drug-induced hepatotoxicity involving denosumab. The time course, the prolonged half-life of the medication and the absence of any other cause implicate denosumab as the offending agent. Causation deals with the question of how a particular clinical event occurs in an individual patient. It is difficult to establish a clear cause-effect relationship. A cause must precede its effect in time. The Naranjo criteria consist of

a standardized case-causality assessment that is well accepted and routine in pharmacovigilance centers around the world. Applying the Naranjo adverse drug reaction probability score to this case, produces a result of 6, which is consistent with a probable drug reaction[15]. This is, despite, the fact that for obvious ethical reasons, the readministration could not be performed. Also, applying Zimmerman's[16] criteria-Hy's Law to estimate severity and the likelihood that Prolia as a therapeutic agent will cause severe hepatocytotoxicity, we considered the blood test combined as an evidence of hepatic injury, e.g., decreased hepatic function, the absence of a chronic liver disease that may induce damage. Hy's law requires elevation of >3 × ULN alanine aminotransferase (ALT) or aspartate aminotransferase (AST) activity; >2 × ULN total bilirubin (TB) (> 1.5 × ULN INR) without a >2 × ULN alkaline phosphatase (ALP). Senior[17] commented that elevation of serum ALT activity is sensitive, but not entirely specific for liver injury, while TB is more specific to the liver but insensitive for decisive liver dysfunction. Together, they have been useful for severe liver toxicity. Hypersensitivity syndrome reactions (HSR) are idiosyncratic host-dependent adverse drug reactions. The mechanism of HSR development is believed to involve a combination of genetic, immunologic and metabolic factors. Genetically susceptible individuals carry defects in drug detoxifying pathways, such that a greater amount of a reactive drug metabolite is produced than that which can be detoxified[18]. The lymphocyte toxicity assay (LTA) is an *in vitro* laboratory tool that can be used to diagnose and predict clinical HSRs. The LTA makes use of lymphocytes isolated from a patient's blood. Lymphocytes are chosen as surrogate target cells as they possess the patient's genotype and will phenotypically express the patient's drug detoxifying deficiencies[6,9]. As lymphocytes do not possess metabolizing capabilities of their own, a murine liver metabolizing system is used, which allows for the toxicities associated with the parent drug and its reactive metabolites to be tested independently. Also, the potential toxicities of related drugs may also be tested, providing an *in vitro* model that can predict possible future clinical reactions. Cellular viability is measured spectrophotometrically. The LTA has been validated for some anticonvulsants, non-steroidal anti-inflammatory drugs, and sulfonamide antibiotics[6,9,10,19-24]. The *in vitro* method described is a noninvasive test by which individual susceptibilities to a drug can be assessed. For a patient with a known serious reaction to a member of a given family of drugs, it is clearly advantageous to be able to select the pharmacologic agent associated with the least amount of risk without exposing that individual to *in vivo* rechallenge. The LTA test is an objective demonstration with scientific value. The patient presented a 31% toxicity when exposed to the monoclonal antibody. Thus, the results we have reported incriminate the antibody as responsible for the idiosyncratic reaction in our patient. Human lymphocytes from patients with suspected HSRs are used as surrogate target cells because they do not contain enzymes that produce the toxic metabolite form of the parent drug, but they do contain the enzymes that are required for detoxification, and if there are genetic deficiencies in these enzymes, these defects are phenotypically expressed in lymphocytes[6]. The cytokine expression in polymorphonuclear cells and their analysis in serum showed elevated levels of several of these molecules in patients, compared to normal individuals. For individuals with DILI, cytokine analysis was performed several years after the latest incident. Neuman and Nicar[24] found that chemoattractant (e.g. interleukin-8) and pro-inflammatory (e.g. TNF-a) cytokines, as well as cell death markers, were several times higher especially in patients with the organ involvement. The reported mean prevalence of osteoporosis ranges from 13%-60% in chronic cholestasis[25]. Primary biliary cholangitis (PBC) is a disease that is typically associated with osteoporosis. There is a report of patients with PBC having low levels of RANKL[26]. Moreover, there are reports showing that in chronic liver disease RANKL has lower levels than controls that do not present liver disease[27.] The RANKL/osteoprotegerin (OPG) system modulates osteoclast activity. OPG inhibits that activity, while the RANKL activates it. OPG/RANKL ratio is high in patients with chronic liver disease compared to control subjects, showing that RANKL activates osteoclastic activity and an excess OPG as a compensating mechanism, which prevents the loss of bone mass[28]. Other cytokines involved in the pathogenesis of chronic liver disease such as interleukin (IL)-1, IL-6 and tumor necrosis factor α can activate this system[29]. Additionally, circulating mononuclear cells could have a higher capacity to differentiate into osteoclasts in patients with chronic liver disease and osteopenia[30]. All of the above observations are in con-

cordance with the fact that by actively reducing RANKL (Table II) denosumab might produce a cholestatic reaction. In analyzing the possible denosumab-induced liver injury, we also took into consideration other factors: the limits of the time interval between the beginning of the treatment with the suspected drug and appearance of the liver injury, the clinical symptoms and laboratory results, as well as the lack of other possible causes on the LTA. We suggest that denosumab induced elevation of other members of the tumor necrosis family, as well as other inflammatory cytokines and chemokines to the range of levels we have previously described in patients with PBC[29]. Due to the prolonged half-life of denosumab (25.4 days), this immune activation is a long-lasting event. Interestingly there is a report in mice of RANKL protecting against hepatic ischemia and reperfusion[31]. In addition to prolonged immune activation, there is a concomitant loss of RANKL due to the direct effect of the denosumab, which deprives the liver of a means of defense. The elevation in serum ALT and AST is related to damage or destruction of the hepatic tissue, rich in the aminotransferases, and/or to changes in cell membrane permeability that permit leakage from the cytosol into the circulation[32,33]. Clearance of the serum aminotransferases requires catabolism by the reticuloendothelial system. The pattern of abnormalities of biochemical tests is routinely clinically employed. For example, elevation of serum aminotransferases indicates hepatocellular injury, while elevation of the total serum bilirubin and alkaline phosphatase indicates cholestasis[16,17,32-35].

Therefore, the patterns of biochemical changes in our patient are consistent with specific drug-induced liver injury that prompted us to specific additional testing and therapeutic intervention6. There is no evidence of disease in humans attributable to denosumab toxicity, other than the observation of hepatic injury reported here.

Conclusions

For patients with DILI induced by medications, early identification and withdrawal of the offending agent improve the prognosis. The long half-life of denosumab makes this extremely difficult to achieve. Any agents that could be causative should be immediately discontinued in the setting of symptoms suggestive of DILI. Patients who survive DILI must be carefully educated about future avoidance and understand that re-exposure may be fatal. Therefore, it is important to test if the patient has any sensitivity to other drugs from the same class[16].

Acknowledgements

The clinical investigation was performed in Kaplan MSC, Rehovot, Israel. All the laboratory analysis have been performed in In Vitro Drug Safety and Biotechnology, Toronto, Canada.

Conflict of interest

The authors declare no conflicts of interest.

References

1) Gerend M, Erchull M, Aiken L MJ. Reasons and risk: factors underlying women's perceptions of susceptibility to osteoporosis. Maturitas 2006; 55: 227-237.
2) Delmas P. Treatment of postmenopausal osteoporosis. Lancet 2002; 359: 2018-2026.
3) Rubin MR, Bilezikian JP. New anabolic therapies in osteoporosis. Curr Opin Rheumatol 2002; 14: 433-440.
4) Black DM, Greenspan SL, Ensrud KE, Palermo L, McGowan JA, Lang TF, Garnero P, Bouxsein ML, Bilezikian JP, Rosen CJ; PaTH Study Investigators. The effects of parathyroid hormone and alendronate alone or in combination in postmenopausal osteoporosis. N Engl J Med 2003; 349: 1207-1215.
5) Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature 2003; 423: 337-342.
6) Neuman MG, Malkiewicz IM, Shear NH. A novel lymphocyte toxicity assay to assess drug hypersensitivity syndromes. Clin Biochem 2000; 33: 517-524.
7) Neuman MG. Cytokines and inflamed liver. Clin Biochem 1999; 33: 601-605.
8) Neuman MG. Apoptosis in diseases of the liver. Crit Rev Clin Lab Sci 2001; 38: 109-166.
9) Neuman M, Nicar M. Apoptosis in ibuprofen-induced Stevens-Johnson syndrome. Transl Res 2007; 149: 254-259.
10) Neuman MG, McKinney KK, Nanau RM, Kong V, Malkiewicz I, Mazulli T, Moussa G. Drug-induced severe adverse reaction enhanced by human herpes virus-6 reactivation. Transl Res 2013; 161: 430-440.
11) Ghermandi R., Terzi S., Gasbarrini A., Boriani S. Denosumab: non-surgical treatment option for selective arterial embolization resistant aneurysmal bone cyst of the spine and sacrum. Case report Eur Rev Med Pharmacol Sci 2016; 20: 3692-3695.

12) Scheiner S, Pivonka P, Smith DW, Dunstan CR, Hellmich C. Mathematical modeling of postmenopausal osteoporosis and its treatment by the anti-catabolic drug denosumab. Int J Numer Method Biomed Eng 2014; 30: 1-27.

13) Boyce BF, Xing L. Functions of RANKL/RANK/OPG in bone modeling and remodeling. Arch Biochem Biophys 2008; 473: 139-146.

14) Ominsky MS, Li X, Asuncion FJ, Barrero M, Warmington KS, Dwyer D, Dwyer D, Stolina M, Geng Z, Grisanti M, Tan HL, Corbin T, McCabe J, Simonet WS, Ke HZ, Kostenuik PJ. RANKL inhibition with osteoprotegerin increases bone strength by improving cortical and trabecular bone architecture in ovariectomized rats. J Bone Miner Res 2008; 23: 672-682.

15) Naranjo CA, Busto U, Sellers EM, Sandor P, Ruiz I, Roberts EA, Janecek E, Domeco C, Greenblatt DJ. A method for estimating the probability of adverse drug reactions. Clin Pharmacol Ther 1981; 30: 239-245.

16) Zimmerman HJ. Hepatotoxicity: the adverse effects of drug and other chemicals on the hepatic, 2nd edition, Philadelphia, Lippincott Williams and Wilkins Publishing, Chapters 4, 5 and 16, 1999.

17) Senior J. How can "Hy's Law" help the clinician? Pharmacoepidemiol Drug Saf 2006; 15: 235-239.

18) Shear NH, Spielberg SP. Anticonvulsant hypersensitivity syndrome. In vitro assessment of risk. J Clin Invest 1988; 82: 1826-1832.

19) Neuman MG, Malkiewicz IM, Phillips EJ, Rachlis AR, Ong D, Yeung E, Shear NH. Monitoring adverse drug reactions to sulfonamide antibiotics in human immunodeficiency virus-infected individuals. Ther Drug Monit 2002; 24: 728-736.

20) Neuman MG, Shear NH, Malkiewicz IM, Kessas M, Lee AW, Cohen L. Predicting possible zonisamide hypersensitivity syndrome. Exp Dermatol 2008; 17: 1045-1051.

21) Neuman MG, Cohen L, Nanau RM Hang P. Genetic and immune predictors for hypersensitivity syndrome to antiepileptic drugs. Transl Res 2012; 159: 397-406.

22) Neuman MG, Shear NH, Malkiewicz IM, Taeri M, Shapiro LE, Krivoy N, Haber J, Gomez M, Fish J, Cartotto R, Cohen L. Immunopathogenesis of hypersensitivity syndrome reactions to sulfonamides. Transl Res 2007; 149: 243-253.

23) Neuman MG, Shear NH, Malkiewicz IM, Lee A, Kesas M, Cohen L. Predicting possible Zonisomide-induced hypersensitivity. Exp Dermatol 2008; 17: 1045-1051.

24) Neuman MG, Nicar M. Ibuprophen-induced toxic epidermal necrolysis. Transl Res 2007; 149: 254-259.

25) Nakchbandi IA, van der Merwe SW. Current understanding of osteoporosis associated with liver disease. Nat Rev Gastroenterol Hepatol 2009; 6: 660-670.

26) Szalay F, Hegedus D, Lakatos PL, Tornai I, Bajnok E, Dunkel K, Lakatos P. High serum osteoprotegerin and low RANKL in primary biliary cirrhosis. J Hepatol 2003; 38: 395-400.

27) Moschen AR, Kaser A, Stadlmann S, Millonig G, Kaser S, Mühllechner P, Habior A, Graziadei I, Vogel W, Tilg H. The RANKL/OPG system and bone mineral density in patients with chronic liver disease. J Hepatology 2005; 43: 973-983.

28) González-Calvin JL, Mundi JL, Casado-Caballero FJ, Abadia AC, Martin-Ibañez JJ. Bone mineral density and serum levels of soluble tumor necrosis factors, estradiol, and osteoprotegerin in postmenopausal women with cirrhosis after viral hepatitis. J Clin Endocrinol Metab 2009; 94: 4844-4850.

29) Neuman M, Angulo P, Malkiewicz I, Jorgensen R, Shear N, Dickson ER, Haber J, Katz G, Lindor K. Tumor necrosis factor-alpha and transforming growth factor-beta reflect severity of liver damage in primary biliary cirrhosis. J Gastroenterol Hepatol 2002; 17: 196-202.

30) Olivier BJ, Schoenmaker T, Mebius RE, Everts V, Mulder CJ, van Nieuwkerk KM, de Vries TJ, van der Merwe SW. Increased osteoclast formation and activity by peripheral blood mononuclear cells in chronic liver disease patients with osteopenia. Hepatology 2008; 47: 259-267.

31) Sakai N, Van Sweringen H, Schuster R, Blanchard J, Burns JM, Tevar AD, Edwards MJ, Lentsch AB. Receptor activity of nuclear factor-kB ligand (RANKL) protects against hepatic ischemia/reperfusion injury in mice. Hepatology 2012; 55: 888-897.

32) Zimmerman HJ, West M. Serum enzyme levels in the diagnosis of hepatic disease. Am J Gastroenterol 1963; 40: 387-404.

33) Ellis G, Goldberg DM, Spooner RJ, Ward AM. Serum enzyme tests in diseases of the liver and biliary tree. Am J Clin Pathol 1978; 70: 248-258.

34) Danan G, Bénichou C, Begaud B, Biour M, Couzigou P, Evreux JC, Lagier G, Berthelot P, Benhamou JP. Criteria of imputation of acute hepatitis to a drug. Results of consensus meetings. Gastroenterol Clin Biol 1987; 11: 581-585.

35) Danan G, Teschke R. RUCAM in drug and herb induced liver injury: the update. Int J Mol Sci 2016; 17. pii: E14.